Epidemiology of Streptococcus suis infection in Viet Nam

Thesis

Epidemiology of *Streptococcus suis* infection in Viet Nam

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Abstract

Human *Streptococcus suis* infection is an emerging zoonotic disease in Southeast Asian countries. It can seriously threaten human heath causing both outbreaks with high morbidity and mortality and endemic disease. It can also have a major negative impact on the economically important pig industry across the region. The aims of this thesis were to understand the epidemiology of human *S. suis* infection in Viet Nam, focusing on the incidence rate, seasonality and risk factors for infection with *S. suis*.

I conducted a prospective surveillance of central nervous system (CNS) infections in twelve provinces of Viet Nam including the Central, the Highlands and the south of the country. I was able to demonstrate that *S. suis* was an endemic disease across southern Viet Nam, and responsible for 49% of adult purulent bacterial meningitis. The incidence rate was 0.57 per 100,000 adult person-years (95% CI, 0.47-0.70) and infection had a case fatality rate of 8%. *S. suis* meningitis tended to predominantly occur in the hottest months of the year in central Viet Nam, but this seasonal pattern was not documented in the south of the country.

A case control study demonstrated that occupational exposure to pigs or pork was a significant risk factor for *S. suis* infection. I was also able to identify novel and important risk factors for *S. suis* disease among the Vietnamese population. Eating fresh blood and undercooked pig products (OR_1 = 2.22; 95%CI = [1.15-4.28] and OR_2 = 4.44; 95%CI = [2.15-9.15]) and exposures of people with skin injuries to pigs or pork (OR = 7.48; 95% CI = [1.97-28.44] and OR_2 = 15.96; 95%CI = [2.97-85.72]), were significant risk factors. These risk factors can be addressed in health education

programs targeted at individuals and communities at risk, focusing on skin protection for those in direct contact with pigs or pork and avoiding eating fresh blood and undercooked pig products. In addition, we were unable to detect *S. suis* serotype 2 DNA from the throat and rectal swab samples of 1523 healthy persons and non-*S. suis* infected patients, including those with exposure to pigs and pork meat. Hence I was not able to demonstrate human carriage of *S. suis* serotype 2 in the Vietnamese population.

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Declaration

Other than the assistance outlined in the acknowledgements, the work described in this thesis is my own work and has not been submitted for a degree or other qualification to this or any other university.

Abbreviations

AFB	Acid fast bacilli
AIDS	Acquired immunodeficiency syndrome
BA	Blood agar
BM	Bacterial meningitis
CA	Chocolate agar
CDC	Centers for Disease Control and Prevention
CFR	Case fatality rate
CI	Confidence interval
CNS	Central nervous system
CSF	Cerebrospinal fluid
CT	Computed tomography
DENV	Dengue virus
DNA	Deoxyribonucleic acid
E/M	Encephalitis/meningitis
EF	Extracellular protein factor
ELISA	Enzyme linked immunosorbent assay
EPI	Expanded Program on Immunization
GAS	Group A Streptococci
GCS	Glasgow Coma Scale
HCMC	Ho Chi Minh City
HIV	Human immunodeficiency virus
HTD	Hospital for Tropical Diseases

IgM	Immunoglobulin M
IGRA	Interferon-gamma release assay
IQR	Interquartile range
IR	Incidence rate
IRR	Incidence rate ratio
JE	Japanese encephalitis
JEV	Japanese encephalitis virus
MAC-ELISA	IgM antibody capture ELISA
MIC	Minimum Inhibitory Concentration
MRI	Magnetic resonance imaging
MRP	Muramidase-released protein
NAAT	Nucleic acid amplification test
NHTD	National Hospital for Tropical Diseases
OD	Optical density
OR	Odds ratio
OUCRU	Oxford University Clinical Research Unit
PBS	Phosphate buffered saline
PCR	Polymerase Chain Reaction
RNA	Ribonucleic acid
RR	Relative risk
SARS	Severe acute respiratory syndrome
TBM	Tuberculous meningitis
TST	Tuberculin skin test

U	Unit
UK	United Kingdom
US	United States of America
WC	White cell count
WHO	World Health Organization

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Chapter 1

Introduction

Emerging infectious diseases, most of which are considered zoonotic in origin, continue to bring about a significant toll on society (Greger 2007). In a review of 1,407 species of human pathogenic organisms, 816 (58%) were classified as zoonotic (Woolhouse et al. 2005). Since the beginning of the new millennium, Asian countries have experienced at least three outbreaks in humans related to emerging zoonotic pathogens. The first outbreak, which happened in 2002-2003 with over 800 deaths, was caused by a new virus, SARS-associated coronavirus, suspected to have originated in bats (Li et al. 2005). After that, human infection with influenzavirus A subtype H5N1, a highly pathogenic avian influenza virus, firstly appeared in the Hong Kong Special Administrative Region in May 1997. This virus has since spread to other countries in the world and has caused major outbreaks in poultry and humans (Ma et al. 2009). A neglected zoonotic pathogen, *Streptococcus suis* serotype 2, caused an explosive outbreak in humans in Sichuan province of China in 2005, with 215 patients and 39 deaths. Prior to this *S. suis* had been reported to cause sporadic cases in humans in some European and Asian countries (Arends et al. 1988; Walsh et al. 1992; Kay et al. 1995; Yu et al. 2006). *S. suis* infections are recognized as a major problem in the swine industry worldwide, after it was first isolated from pigs in 1956 (Arends et al. 1988; Gottschalk et al. 2007). It is not a new pathogen in humans but the Chinese outbreak significantly increased the awareness of this bacterium in the medical community because of its high morbidity and mortality. In Viet Nam, *S. suis* meningitis in humans was first reported in 1996 (Mai et al. 2008). The number of

human cases has increased annually and it is now the most common pathogen of acute adult bacterial meningitis in the south of Viet Nam (Mai et al. 2008). To date little is known of the epidemiology of human *S. suis* infection in Viet Nam, including risk factors, and my thesis will focus on this aspect of the infection.

1.1 Epidemiology

1.1.1 *Streptococcus suis* infection in pigs

1.1.1.1 Prevalence of *Streptococcus suis* infection in pigs

S. suis can be a pathogen or a commensal in the respiratory, alimentary and reproductive tracts of pigs. The prevalence of asymptomatic or symptomatic infection of *S. suis* varies among herds (Staats et al. 1997). Although the pig carrier rate can reach up to 100%, the incidence of disease varies over time and affects generally less than 5% of a herd (Clifton-Hadley et al. 1986). Pigs can carry multiple serotypes in their tonsils, but serotype 2 is most often related to serious disease. Outbreaks caused by *S. suis* serotype 2 occur predominantly in piglets between 4 to 12 weeks of age. The peak of disease happens during the weaning period (about 6 weeks of age) and after mixing of pigs. Pigs at any age can be affected but susceptibility generally decreases with age following weaning (Lamont et al. 1980; Clifton-Hadley et al. 1984; Reams et al. 1993). There may be an association between infection with porcine reproductive and respiratory syndrome virus and increasing susceptibility of pigs to *S. suis* infection. However, more field research is needed to confirm this hypothesis (Thanawongnuwech et al. 2000; Feng et al. 2001; Xu et al. 2010). In addition to pigs, *S. suis* can be also isolated from other animals, such as ruminants, cats, dogs, deer, and horses and is believed to be commensal in the intestinal flora (Staats et al. 1997).

1.1.1.2 Transmission of *Streptococcus suis* among pigs

S. suis usually colonizes the palatine tonsils, nasal cavity, alimentary and reproductive tract of subclinical carriers. Carriers of *S. suis* are nasally or orally infectious to other pigs. The transmission of bacteria between herds usually occurs by the movement of healthy carrier pigs (Marois et al. 2007). That means infection is more likely at weaning when they are moved, mixed and housed in crowded conditions. Sows are also another probable source of infection. Gilts and sows may harbour *S. suis* in their uterus or vagina and transmit the bacteria to infant piglets after early contact (Staats et al. 1997; Huang et al. 2005; Sriskandan et al. 2006; Marois et al. 2007). However, the carrier pigs are not the sole source of infection. The other sources may be the contamination of the environment, personnel or materials. *S. suis* serotype 2 is an important contaminant of faeces, dust and water. It can survive in water for 10 minutes at 60 ^{0}C and for 2 hours at 50 ^{0}C, whereas at 25 ^{0}C, the organism can survive for 24 hours in dust and for 8 days in faeces. Vectors of *S. suis* can also play a role in disease transmission in pigs and include houseflies and mice (Staats et al. 1997).

1.1.1.3 Manifestations of disease in pigs

In acute cases, pigs may die within hours of the onset of clinical signs but it is not uncommon for death to occur without apparent clinical signs. Meningitis is the most striking feature and often the basis of a presumptive diagnosis. Other manifestations of *S. suis* infection are arthritis, endocarditis, pneumonia, rhinitis, abortion and vaginitis (Windsor et al. 1975; Gottschalk et al. 2007). In the acute forms of the disease, the clinical signs may include fever, depression, anorexia, and lassitude, followed by one or more of the following: ataxia, incoordination, tremors,

opisthotonus, blindness, loss of hearing, paddling, paralysis, dyspnoea, convulsions, nystagmus, arthritis, lameness, erythema and abortion (Staats et al. 1997).

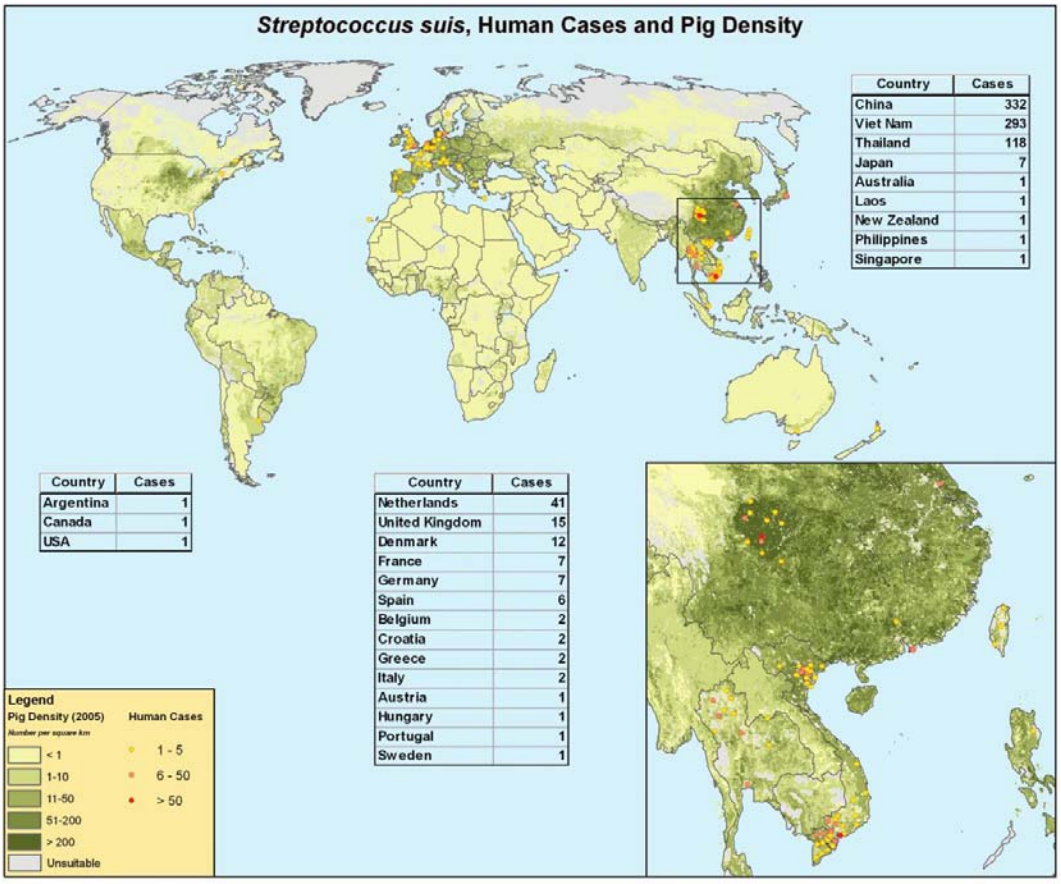
1.1.2 *Streptococcus suis* infection in humans

1.1.2.1 *Streptococcus suis* infection in human situation

Streptococcus suis infection is known to occur sporadically in humans, in intensive pig rearing countries, without an obvious seasonal pattern (Gottschalk et al. 2007). The first human case was reported in Denmark in 1968 (Perch et al. 1968). After that, this infection was also documented in other European countries, including 30 cases in the Netherlands in a period between 1969 and 1984, 35 cases in England from 1975 to 1990, 7 cases in France, and 4 cases in Germany. Figure 1-1 presents the world map of human *S. suis* cases with background pig density data (Arends et al. 1988; Walsh et al. 1992; Kay et al. 1995; Wertheim et al. 2009).

Figure 1-1 World map of human *S. suis* cases with background pig density data

(Wertheim et al. 2009)



In Asia, human *S. suis* infection had mainly been reported in Hong Kong, where 61 cases occurred in the period of 1981 – 1993, and in Thailand with 9 cases (Chau et al. 1983; Kay et al. 1995; Leelarasamee et al. 1997). However, the number of human cases in Asian countries reported in the medical literature sharply increased after the serious outbreak in Sichuan province of China in July 2005 (Hui et al. 2005; Wangkaew et al. 2006; Yu et al. 2006; Mai et al. 2008; Wangsomboonsiri et al. 2008; Wertheim et al. 2009). It is difficult to prove whether Asian human cases have truly increased since the year 2005. One hypothesis is that the 2005 outbreak significantly increased not only the interest of the scientific world in this poorly known pathogen,

but also the awareness of this infection in humans. As a consequence, *S. suis* diagnostics was probably improved in Asian countries (Gottschalk et al. 2010).

While most human *S. suis* were reported as sporadic cases, three outbreaks in humans have been reported. These include the outbreak in China mentioned above, with 215 cases and 39 deaths, a smaller outbreak in Jiangsu provinces of China in 1998 with 25 cases and 14 deaths, and a recent outbreak in Thailand with 49 cases and 3 deaths (Yu et al. 2006; Lun et al. 2007; Khadthasrima et al. 2009).

1.1.2.2 Risk factors of *Streptococcus suis* infection in humans

S. suis infection was usually described in middle-aged males, who had an occupational exposure to pigs or pork. The relatively high mean patient age (47-55 years of age) as well as the high male-to-female patient ratio (which ranged from 3.5 to 6.5) supports the notion that infection with *S. suis* is generally an occupational disease in which males predominate in the work force (Arends et al. 1988; Walsh et al. 1992; Kay et al. 1995; Wangkaew et al. 2006; Yu et al. 2006; Mai et al. 2008). In the Netherlands, the annual risk of developing *S. suis* meningitis among abattoir workers and pig breeders was estimated at 3.0 per 100,000 people; the risk was lower for butchers, at 1.2 per 100,000 people. This rate was 1,500 times higher than the rate among general population (not working in the swine industry), which was 0.002 per 100,000 people (Arends et al. 1988). However, the risk of infection appears dramatically higher in Asian countries. The rates were 0.09 per 100,000 in general population and 32 per 100,000 in population having occupations related to pigs or pork in Hong Kong, China (Ma et al. 2008). Such an estimate has not been made for the Southeast Asia, where the pig density is high.

It is hypothesized that patients may be infected through minor cuts or abrasions on their skin when exposed to pork or pigs (Arends et al. 1988; Walsh et al. 1992). Handling diseased pigs certainly increases the risk of human infection. In a matched case-control study of risk factors for human *S. suis* infection during the outbreak in Sichuan province of China, slaughtering (OR, 11.9; 95%CI, 3.4-42.8) and cutting carcasses and processing sick or dead pigs (OR, 3.0; 95%CI, 1.0-8.8) were important independent risk factors for human infection (Yu et al. 2005). However, whilst occupational exposure to pigs or pork was documented in 88% of European patients, it was reported in less than 50% of Asian cases, suggesting the contribution of other behavioural or exposure related risk factors in the Asian population (Kay et al. 1995; Mai et al. 2008). Asian farmers often share their accommodation with pigs, and it is common practice for diseased animals to be slaughtered at home and consumed (Sriskandan et al. 2006). In addition, case series from Hong Kong and Viet Nam included not only a significant number of housewives, presumably infected as a result of contact with contaminated pork, but also other individuals who could not recall any exposure to pigs or pork prior to their illness (Kay et al. 1995; Mai et al. 2008). Asian consumers prefer to buy fresh pork from wet markets, where animals are usually caged and killed for live preparation and its products are generally stored for short periods of time and are always expected to be fresh. *S. suis* was isolated from 6.1% of raw pork meat from 3 of the 6 wet markets in Hong Kong (Ip et al. 2007). Consequently, it is hypothesized that processing or consuming uncooked or partial cooked pork products is also a risk factor for *S. suis* infection. Local delicacies, such as undercooked pig tonsils, intestines, uterus, and fresh pig blood, may also represent important sources of infection. More research is needed to elucidate this hypothesis.

Skin lesions, the suspected portal of entry in human cases, were documented in 15-50% of *S. suis* infection patients (Figure 1-2) (Arends et al. 1988; Walsh et al. 1992; Kay et al. 1995; Yu et al. 2006). Many patients had no history of skin injury at all, and the respiratory or oral route of infection remains a possibility. The latter scenario might explain why diarrhoea was one of the present symptoms documented in about 15% of patients (Kay et al. 1995).

Although asymptomatic carriage of *S. suis* is common in healthy pigs, it is unknown whether human carriage is common. This status could potentially contribute to an increased risk of infection, and to the possibility of person-to-person transmission. Subclinical infection may also occur in humans. None of 16 veterinarian students, but 9/96 (9%) of dairy farmers, 11/107 (10%) of meat inspectors, and 15/70 (21%) of pig farmers were seropositive to *S. suis* serotype 2 in a study in New Zealand (Robertson et al. 1989). Nasopharyngeal carriage of *S. suis* was studied in a group at high risk of infection (butchers, abattoir workers and meat processing employees) in Germany. Carriage rate was reported as 5.3% (7/132) of high risk group, compared to 0% (0/130) of a control group (Strangmann et al. 2002). These findings indicate that *S. suis* carriage does occur in individuals with prolonged and recurrent exposure to pigs and pork. However, the prevalence, duration and importance of *S. suis* carriage in humans are unknown.

Figure 1-2 Small cuts on the second digit of a butcher presenting with *S. suis* meningitis

1.2 Clinical aspects of *Streptococcus suis* infection in humans

In humans, the most common clinical manifestation of *S. suis* infection is purulent bacterial meningitis (Arends et al. 1988; Kay et al. 1995; Mai et al. 2008). The majority of patients presented with symptoms and signs of bacterial meningitis, including fever, headache, neck stiffness and CSF leukocytosis (Mai et al. 2008; Wertheim et al. 2009). A striking feature, which is indicative of *S. suis* infection in a Vietnamese middle-aged patient presenting with signs and symptoms of meningitis, is the presence of unilateral or bilateral hearing loss accompanied with tinnitus and vestibular dysfunction on or shortly after admission. Hearing loss is the most common

sequel of *S. suis* meningitis, which was reported in 50-80% of *S. suis* meningitis patients, compared to 20-30% of *S. pneumoniae* meningitis patients (Arends et al. 1988; Walsh et al. 1992; Kay et al. 1995; Mai et al. 2008). Cochlear sepsis, resulting from passage of the organism from the subarachnoid space to the perilymph via the cochlear aqueduct, might be primary responsible for the hearing loss (Kay 1991). The mortality of *S. suis* meningitis ranges between 1% (1/102) in China and 7% (2/30) in the Netherlands (Arends et al. 1988; Kay et al. 1995; Yu et al. 2006; Mai et al. 2008)

S. suis infection may also present with septic shock syndrome, similar to other encapsulated microorganisms which cause meningitis, such as *Streptococcus pneumoniae* and *Neisseria meningitidis*. Findings include widespread skin lesions, ranging from petechia or purpura to large hemorrhages with central necrosis and/or conjunctival hemorrhages (Figure 1-3). Necrotic fingers or toes may be present in very severe cases (Figure 1- 4). The mortality rate in patients presenting with septic shock case was over 60% while that of meningitis was less than 10% (Arends et al. 1988; Leelarasamee et al. 1997; Yu et al. 2006; Mai et al. 2008). The presence of septic shock complicating *S.* suis infection varies in the published literature from 2/151 (1%) in southern Viet Nam, 6/50 (12%) in the north of Viet Nam and 61/215 (28%) during the outbreak in the Sichuan province of China (Yu et al. 2006; Mai et al. 2008; Wertheim et al. 2009). The reason of this difference is unknown but it may be explained by differences in virulence of pathogen, in bacterial load of infection, by different host genetic susceptibility, as well as by delay in presenting to hospital. In the outbreak report from China, septic shock cases met the case definition of streptococcal toxic shock syndrome caused by group A streptococci (GAS) established by the Centers for Disease Control and Prevention (CDC). However,

superantigens were not detected in these bacterial isolates. The exact mechanism by which *S. suis* serotype 2, a non-GAS, causes septic shock syndrome is yet to be determined (Stevens 1995; Tang et al. 2006).

Other less common clinical manifestations of *S. suis* infection are endocarditis, arthritis, spondylodiscitis, cellulitis, pneumonia, peritonitis, and endophthalmitis (Walsh et al. 1992; Vilaichone et al. 2000; Wangkaew et al. 2006). In a series of 41 cases from Chiang Mai University Hospital, infective endocarditis accounted for 39% (16/41) of patients. Onset of illness in 12 patients was longer than 1 week. Only 3 patients had underlying heart diseases and no patient was an intravenous drug user (Wangkaew et al. 2006). A striking feature of the case series in UK was high incidence of arthritis, which was reported in 10 of 20 patients having available clinical details. Arthritis was usually suppurative and affected hips, knees, elbows and spine. Pus cells were found in joint fluids but there was only one report of successful isolation of *S. suis* from a human joint aspirate (Walsh et al. 1992).

Figure 1-3 Hemorrhagic rashes in a septic shock syndrome patient with *S.suis*

Figure 1-4 Necrotic toes in a septic shock syndrome patient with *S.suis*.

1.3 Microbiological aspects of *Streptococcus suis*

1.3.1 Lancefield group and serotypes of *Streptococcus suis*

Streptococcus suis is a Gram-positive facultative anaerobe, whose composition of the capsule defines 35 serotypes (Higgins et al. 1995). Initially, it was ascribed to Lancefield groups R, S, RS and T by de Moor (de Moor 1963). After that, researchers discovered that lipoteichoic acid extracted from cell wall of *S. suis*, reacted with group D antiserum (Elliott et al. 1977) and de Moor probably worked with antigens extracted from the capsular material rather than from the cell wall (Gottschalk et al. 2007). Lancefield groups S, R, RS and T were then reclassified as capsular types 1, 2, 1/2 and 15, respectively, according to immunological specificity of capsular polysaccharides (Elliott et al. 1978; Gottschalk et al. 1989). Some laboratories used the results of multi-test, such as API Strep system test (BioMérieux, France), to differentiate *S. suis* serotypes 1 and 2 based on biochemical properties, erroneously, since these tests only differentiate biotypes (e.g. I and II in API Strep). Serotyping is still an important part of the routine diagnostic procedure (Gottschalk 2004). Though *S. suis* cell wall antigen shares epitopes with Lancefield group D, negative reactions are often observed with some strains and it is not closely related genetically to other members of this group, such as enterococci (Chau et al. 1983; Tarradas et al. 2001; Gottschalk et al. 2007). Recently, analysis of the sequences of 16S rRNA and *cpn*60 genes of 35 serotypes of *S. suis* found that serotypes 32 and 34 may present a distinct species, *Streptococcus orisratti* (Brousseau et al. 2001; Hill et al. 2005). Though other serotypes, such as serotype 1, 4, 14 and 16, were sporadically reported in human cases, serotype 2 is the most common serotype causing invasive diseases in human as well as in pigs. For example, 150/151 *S. suis* meningitis cases in

the Vietnamese case-series study and the largest human outbreak were caused by this serotype. According to a recent report from Thailand, 12/177 (6.8%) of human isolates of *S. suis* were belonged to serotype 14, which may be the second most common serotype in Southeast Asian countries (Kopic et al. 2002; Yu et al. 2006; Mai et al. 2008; Nghia et al. 2008; Kerdsin et al. 2009).

1.3.2 Identification of *Streptococcus suis*

Until March 2009, over 750 human cases of *S. suis* infection were reported in the medical literature mainly from Asian and European countries (Wertheim et al. 2009). However, this number is likely to represent an underestimate because the bacteria are often only diagnosed as Gram positive cocci and not further typed or misidentified as *Streptococcus pneumoniae,* α-haemolytic or viridans streptococci, *Enterococcus faecalis,* group D enterococci and *Streptococcus bovis* (Arends et al. 1988; Kay et al. 1995).

S. suis colonies, which are small, greyish or transparent, and slightly mucoid, are α-haemolytic on sheep blood agar plates and are β-haemolytic on horse blood agar (Staats et al. 1997). Four tests are used for a presumptive identification of *S. suis* in a laboratory: no growth in 6.5% NaCl agar, a negative Voges-Proskauer (VP) test, and production of acid in trehalose and salicin broths. However, biochemical characteristics are so variable that identification is often difficult and may require a combination of biochemical reactions followed by confirmational serotyping. For instance, *S. suis* was originally considered negative for mannitol fermentation. However, over 70% of serotype 17, 19 and 21 tested positive (Higgins et al. 1990). Serotyping based on polysaccharides capsular antigens uses one or more of the following techniques: a slide agglutination test, a capsular reaction, a capillary

precipitation or a co-agglutination test (Staats et al. 1997). As a rule of thumb, microbiological laboratories should consider *S. suis* if optochin-resistant streptococci are cultured from a CSF sample obtained from a patient with meningitis (Wertheim et al. 2009). Many laboratories propose the use of multi-tests in identification of *S. suis*, such as API Strep System test (BioMérieux, France), the BBL Crystal Gram-positive ID kit (Becton-Dickinson, NJ, USA), the Vitek GPI Card (BioMérieux) and the Phoenix System PID (Becton-Dickinson) but some *S. suis* strains can be misidentified when using these commercial kits (Gottschalk et al. 2010).

1.3.3 Antimicrobial susceptibility of *Streptococcus suis*

Streptococcus suis strains isolated from pigs were still susceptible to penicillin, ampicillin, third generation cephalosporin drugs but highly resistant to tetracycline before the year 2000 (Table 1-1). The increased use antimicrobial drugs in the swine industry have led to a decrease in the antibiotic susceptibility of *Streptococcus suis* isolates. In a survey on clinically healthy sows at nine different regions in China, proportions of penicillin-, ampicillin- and ceftiofur-resistance *S. suis* isolates were 9.5 % (40/421), 4% (17/421) and 22.1% (93/421), respectively (Zhang et al. 2008). Similarly, in another study in northern Thailand, 27% (14/52) strains resistant to penicillin G, 23% (13/52) strains resistant to ampicillin and 0% (0/52) strain resistant to ceftiofur were reported (Lakkitjaroen et al. 2011). However, differences in antimicrobial susceptibility of *S. suis* isolates from country to country may reflect true regional difference but may also, to some extent, be the result of methodological variations (Han et al. 2001). For example, by disk diffusion method using Mueller-Hinton Agar II supplemented with sheep blood, 39% (53/135) of *S. suis* strains showed intermediate susceptibility of penicillin (inhibition zone diameter

<29 mm) while these strain were completely susceptible to penicillin G by microdilution method (Marie et al. 2002). In addition, the breakpoints used to define susceptible and resistance ranges may vary between studies. Hence, the results from northern Thailand should be cautiously interpreted because the authors used the disk diffusion method.

The decreased antimicrobial susceptibility of *S. suis* strains isolated from pigs raises a considerable concern about human infection with resistant strains, especially strains resistant to β-lactam class of antimicrobials. All of reported strains isolated from humans were completely susceptible to penicillin G, ceftriaxone and vancomycin, except one strain from a Thai patient with peritonitis, which had an MIC to penicillin G greater than 32 μg/ml. We also did not document any *S. suis* strain isolated from CSF samples of 175 Vietnamese meningitis patients which was resistant to these antibiotics (Vilaichone et al. 2002; Wangkaew et al. 2006; Mai et al. 2008; Wertheim et al. 2009; Hoa et al. 2011). However, monitoring of antimicrobial susceptibility should be done regularly for timely detection of resistance trends of *S. suis* in the context of increased antibiotic resistance of this pathogen in the pig population.

Table 1-1 Antimicrobial susceptibility of *Streptococcus suis* strains isolated from pigs

Antimicrobial agents	Minimal inhibitory concentration (MIC) values (μg/ml)							
	USA[1] (n=50)		European countries[2] 1987 – 1997 (n=384)		Japan[3] 1987 – 1996 (n=689)		China[4] 2005 – 2007 (n=421)	
	Range	MIC_{90}[6]	Range	MIC_{90}[6] (%R)[5]	Range	MIC_{90}[6] (%R)[5]	MIC_{50}[6]	MIC_{90}[6] (%R)[5]
Ceftiofur	≤0.03 – 1.0	0.13	0.03 – 0.13	0.03 (0.0)	NA	NA	0.24	8 (22.1)
Penicillin	NA	NA	0.13 – 2.0	0.13 (0.0)	0.0125 – 25	0.2 (0.9)	0.12	2 (9.5)
Ampicillin	≤0.03 – 8.0	0.06	NA	NA	0.0125 – 25	0.1 (0.6)	0.12	2 (4.0)
Tetracycline	0.5 – >32.0	>32.0	0.13 – >64.0	64.0 (75.0)	0.1 - >100.0	50 (86.9)	>8	>8 (91.7)
Enrofloxacin	≤ 0.03 – 0.5	0.5	0.06 – 1.0	0.5 (0.0)	NA	NA	1	4 (32.8)
Chloramphenicol	NA	NA	0.5 – 4.0	2.0 (0.0)	NA	NA	4	64 (19.9)
Spectinomycin	4.0 – >128.0	32.0	2.0 – >128.0	16.0 (3.6)	NA	NA	NA	NA
Trimethoprim-Sulfamethoxazol	≤0.015 – 0.25	0.13	0.06 – 16.0	2.0 (16.0)	<0.025 – 3.12	1.57 (0)	16/304	>16/304 (59.1)

[1] (Salmon et al. 1995)
[2] (Wisselink et al. 2006)
[3] (Kataoka et al. 2000)
[4] (Zhang et al. 2008)
[5] Proportion of resistant strains
[6] MIC $_{50}$ or MIC_{90} means the growth of 50% or 90% strains, respectively, tested was inhibited at this drug concentration.

1.3.4 Virulence of *Streptococcus suis*

Apart from Canada where prevalence of *S. suis* serotype 2 strains isolated from diseased pigs was less than 25%, most of the pathogenic strains in European and Asian countries belonged to serotype 2 (Higgins et al. 2001). Human infection with *S. suis* has been rarely reported in North America, where diseased pigs are commonly documented. Up to 2010, there were only 3 cases in United States and 3 cases in Canada (Trottier et al. 1991; Willenburg et al. 2006; Lee et al. 2008; Fittipaldi et al. 2009; Haleis et al. 2009). However, serological studies suggest that infection may be underreported in the USA (Smith et al. 2008). Taking together, serotype 2 strains may have high virulence and plays an important role in human infection with *S. suis*. It may be hypothesized that Euroasian and North American serotype 2 strains possess a different virulence potential (Gottschalk et al. 2000).

Most studies on the virulence of *S. suis* have focused on serotype 2 strains. A series of potential virulence factors have been identified, including the capsular polysaccharide, extracellular protein factor, muramidase-released protein, suilysin, several adhesins, hyaluronate lyase, surface antigen one (Sao) (Vecht et al. 1991; Jacobs et al. 1994; Smith et al. 1999; Allen et al. 2004; Li et al. 2006; Gottschalk et al. 2007; Esgleas et al. 2008; Zhang et al. 2009). With exception of the capsular polysaccharide, none of these were shown to be essential for virulence in animal models of infection. For example, phenotype MRP^+ EF^+ was presented in 77% (86/111) strains isolated from diseased pigs in a European country while phenotype MRP^- EF^- was reported in 77% (44/57) strains isolated from diseased pigs in Canada (Vecht et al. 1991; Gottschalk et al. 1998). In the case series from Viet Nam, 92/92 (100%) of *S. suis* strains isolated from meningitis patients were *sly* positive. Other

virulence factors, such as *epf*, *epf**, and MRP production, were present in 50% (46/92), 50% (46/92) and 70% (64/92) of strains, respectively. Infection with a strain carrying the *epf* gene was independently associated with severe deafness at discharge (p=0.045) (Mai et al. 2008)

Two Chinese outbreak isolates were fully sequenced, and a proposed pathogenicity island, which may have been involved in the particular clinical manifestations observed during the outbreak in 2005, was identified (Chen et al. 2007). Further bioinformatics analysis revealed a unique two-component signal transduction system that may be essential for full virulence of the highly invasive Chinese strains (Li et al. 2008). A Chinese outbreak strain was also sequenced along with a Vietnamese strain isolated from a human patient, not related to any outbreak, and a European reference strain isolated from a pig. Strains were shown to be highly similar and the Chinese and Vietnamese strain shared many of the genes on the pathogenicity island (Holden et al. 2009). More research is clearly needed to elucidate the bacterial factors that confer virulence in strains of *S. suis*.

1.4 Treatment of *Streptococcus suis* infection

1.4.1 Antimicrobial treatment

Although most *S. suis* strains isolated from humans were sensitive to penicillin G, ampicillin, third-genenation cephalosporin (either ceftriaxone or cefotaxime) and vancomycin (Section 1.1.3), empirical antimicrobial therapy of community acquired meningitis should include third-generation cephalosporin with or without vancomycin to cover penicillin-resistant pneumococci. Reasons for this are the nonspecific manifestations of *S. suis* meningitis and the high antibiotic resistance of *S.*

pneumoniae in Asia. The prevalence of nonsusceptible *S. pneumoniae* strains in Asia, which were isolated from clinical specimens of patients with community-acquired pneumococcal diseases, such as blood, CSF, lower respiratory tract specimens, sinus and middle ear aspirates, exceeds 50% and MIC_{90} of penicillin of these strains was 2.36 μg/ml (Song et al. 2004; Nudelman et al. 2009). Penicillin G or ampicillin can be used in the treatment of *S. suis* infection after results of culture and antimicrobial susceptibility have become available. Duration of antibiotic treatment varied from 2 to 4 weeks according to clinical manifestations (Arends et al. 1988; Kay et al. 1995; Vilaichone et al. 2002; Wangkaew et al. 2006; Mai et al. 2008).

1.4.2 Adjunctive dexamethasone in bacterial meningitis treatment

In a randomized, double-blind, placebo-controlled clinical trial in Viet Nam, dexamethasone (0.4mg/kg twice daily for 4 days) resulted in a significant reduction in the risk of death at 1 month (relative risk, 0.43; 95%CI, 0.02-0.94) and in the risk of death and disability at 6 months (OR, 0.56; 95%CI, 0.32-0.98) in patients with confirmed bacterial meningitis. In the group of *S. suis* meningitis patients, 20/53 (38%) of patients receiving placebo were deaf in at least 1 ear, compared to 7/57 (12%) of patients given dexamethasone (RR, 0.33; 95%CI, 0.15-0.71) (Nguyen et al. 2007). In a multivariate analysis, severe hearing loss was associated with older age (>50 years of age), infection with a strain carrying the *epf* gene and in those not receiving steroid treatment (Mai et al. 2008) Dexamethasone is now given to all adults patients with acute bacterial meningitis in Viet Nam.

1.5 Prevention

1.5.1 Vaccine

A lot of trials of vaccines in pigs were conducted using inactivated bacteria, live avirulent strain or some virulence factors, such as purified capsular polysaccharides, suilysin, muramidase-released protein, extracellular factor and surface antigen one (Elliott et al. 1980; Holt et al. 1990; Jacobs et al. 1996; Busque et al. 1997; Wisselink et al. 2001; Li et al. 2007). However, there are no effective vaccines available for swine because the adaptive (humoral) immune response against *S. suis* is low (data from mice and pigs). The reasons for this are unknown and require further research (Gottschalk et al. 2010). No vaccine is in development for humans.

1.5.2 Other preventive measures

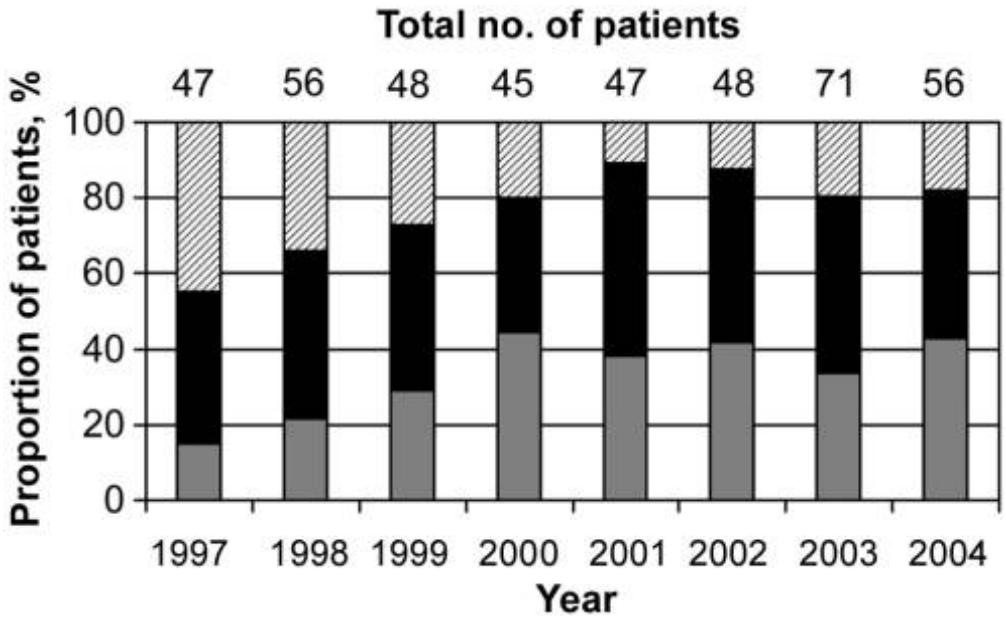
Though *S. suis* can survive in the environment, it is rapidly inactivated by disinfectants and cleansers, commonly used in farms and laboratories (Clifton-Hadley et al. 1984). Several previous studies have shown that *S. suis* infection in human might be related to occupational exposure to pigs or pork (Arends et al. 1988; Walsh et al. 1992; Kay et al. 1995). Individuals in close occupational contact with pigs or pork should pay special attention to protective measures, such as wearing gloves and boots and to disinfect themselves, for example hand washing with disinfectants, before leaving. Individuals who have had a splenectomy should be excluded from the meat trade or pig farms because of the high morbidity and mortality rate reported in these patients following infection with capsulated bacteria including *S. suis* (Gallagher 2001). However, occupational exposure to pigs or pork was reported in less than 50% of Asian patients while eating habit related to raw or undercooked pork

and fresh blood is a local custom in these countries (Kay et al. 1995; Mai et al. 2008; Khadthasrima et al. 2009). Hence, the medical authorities of these countries recommend people avoid slaughtering and eating meat from diseased pigs. We need more epidemiological studies on the risk factors in Asian countries for effective prevention of *S. suis* infection in humans.

1.6 Epidemiological studies on *Streptococcus suis* infection in Viet Nam

The first case series of 151 Vietnamese *S. suis* meningitis cases admitted to a referral hospital for infectious diseases in the south of Viet Nam from 1996 to 2005, was published in 2008. A striking feature of this report was that *S. suis* was identified as the most common pathogen of acute adult bacterial meningitis, and accounted for approximately 34% (151/450) of meningitis cases (Figure 1-5) (Mai et al. 2008). Recently, another case-series from a referral hospital for infectious diseases in the north of Viet Nam revealed that 50/57 (88%) confirmed adult bacterial meningitis cases were caused by *S. suis* while *S. pneumoniae* was only isolated from 3 patients (Wertheim et al. 2009). It is difficult to conclude from these data that *S. suis* infection is really an endemic disease across Viet Nam because these tertiary hospitals received patients with severe central nervous sytem (CNS) infection referred from provincial hospitals. Therefore, additional studies on the aetiology of Central Nervous System (CNS) infection in other provinces of Viet Nam are needed to confirm the true extent of endemicity of human *S. suis* in the country.

Figure 1-5 Annual proportion of adult patients admitted to HTD (a referral hospital for infectious diseases) with *S. suis* meningitis[1] (Mai et al. 2008)



[1] *S. suis* meningitis (gray); other bacterial causes of acute adult meningitis (black); and unconfirmed bacterial meningitis (dashed)

There are also no data on disease incidence in pigs or on the most common serotypes causing disease in pigs in Viet Nam. In a survey of *S. suis* carriage in healthy slaughterhouse pigs in six southern provinces of Viet Nam and the Ho Chi Minh City metropolitan area, 41% (222/542) of pigs carried *S. suis* of one or multiple serotypes. Approximately 8% (45/542) of healthy pigs carried *S. suis* serotype 2, which was the most common serotype reported (Hoa et al. 2011).

1.7 Summary

After carefully reviewing medical literature on *Streptococcus suis* infection in humans, I had the following comments:

1. *S. suis* can seriously threaten human health causing both outbreaks with high morbidity and mortality and endemic disease in humans in certain geographical regions.
2. The number of human cases reported in medical literature has sharply increased in Asian countries, especially in the Southeast Asia. *S. suis* serotype 2 was documented as one of the most common pathogens of purulent bacterial meningitis in Hong Kong and Viet Nam. However, this pathogen was only reported at two referral hospitals for infectious diseases in Ha Noi and Ho Chi Minh City. More research was needed in rural provinces to investigate whether *S. suis* infection are endemic across Viet Nam.
3. Information related to the epidemiological characteristics of *S. suis* infection in Viet Nam is scarce. What is the population at risk in Viet Nam? What is the incidence rate of *S. suis* infection in Viet Nam? How does the incidence rate vary according to pig density or age groups?
4. Recently, reports from China and Thailand showed that *S. suis* strains isolated from pigs had reduced susceptibility to penicillin G and third-generation cephalosporin (ceftiofur). Are these antimicrobial resistance patterns present in *S. suis* strains isolated from humans in Viet Nam?
5. From case series in Viet Nam as well as in other Asian countries, occupational exposure to pigs or pork, the main risk factor in Western countries, was

reported in less than 50% of patients. Are culinary habits or close proximity of pigs within households important risk factors in the Vietnamese patients?

6. Although asymptomatic carriage of *S. suis* is common in healthy pigs, it is unknown whether human carriage is common. This status could potentially contribute to an increased risk of infection, and to the possibility of person-to-person transmission.

1.8 Focus, Aims and Structures of the Thesis:

This thesis examines human *Streptococcus suis* serotype 2 infection in Viet Nam, and aims to address the following questions:

1. Is *Streptococcus suis* serotype 2 infection really a human endemic disease in Viet Nam?
2. What are the epidemiological characteristics of human *Streptococcus suis* serotype 2 infection in Viet Nam? With a focus on seasonal patterns of infection, incidence rate of disease and antimicrobial susceptibility of bacterial strains isolated from humans.
3. What are the important risk factors for human *Streptococcus suis* serotype 2 infection in Viet Nam?
4. Is there human carriage of *Streptococcus suis* serotype 2 in the respiratory and alimentary tracts?

I designed two studies to address the research questions presented above. The first study was a prospective descriptive surveillance study of central nervous system infections in central and southern Vietnam (designated 01SS). The results of this study are described in Chapter 3 and Chapter 4. The second study was a case-control study to identify risk factors of *S. suis* infection in patients presenting with *S. suis*

meningitis and to investigate whether there is human carriage of *S. suis* (designated EN study). This study is described in Chapter 5 and Chapter 6. The setting and methods used for these studies are described in Chapter 2.

Chapter 2

Materials and Methods

2.1 Introduction

This chapter describes the research setting, including geographical regions, population structure, health service and study hospitals. The general clinical research and laboratory methods are also described.

2.2 Setting

2.2.1 Geography of Viet Nam

Viet Nam is located on the Indochinese peninsula in Southeast Asia, between North latitudes $8^0$27' and $23^0$23' and between East longitudes 102^0 and 110^0. It is bordered by China to the north, Laos to the northwest, Cambodia to the southwest and the "Biển Đông" (East Sea) to the east. Its area is approximately 331,051.4 km^2. Viet Nam, which lies in the tropical zone, has 2 major climate regions. Northern Viet Nam (north of Hai Van Mountain) has a climate with four distinguishable seasons, spring, summer, autumn and winter. Southern Viet Nam (southward from Hai Van Mountain) has a rather moderate tropical climate with a dry and a rainy season. Viet Nam is administratively divided into 58 provinces and 5 centrally-administered cities, including Ha Noi, Hai Phong, Da Nang, Ho Chi Minh and Can Tho, which belong to 6 social-economic regions: Red river delta (Ha Noi, Vinh Phuc, Bac Ninh, Quang Ninh, Hai Duong, Hai Phong, Hung Yen, Thai Binh, Ha Nam, Nam Dinh and Ninh Binh); Northern Midlands and Mountain areas (Ha Giang, Cao Bang, Bac Kan, Tuyen Quang, Lao Cai, Yen Bai, Thai Nguyen, Lang Son, Bac Giang, Phu Tho, Dien Bien,

Lai Chau, Son La and Hoa Binh); North Central and Central Coast areas (Thanh Hoa, Nghe An, Ha Tinh, Quang Binh, Quang Tri, Thua Thien – Hue, Da Nang, Quang Nam, Quang Ngai, Binh Dinh, Phu Yen, Khanh Hoa, Ninh Thuan and Binh Thuan); Central Highlands (Kon Tum, Gia Lai, Dak Lak, Dak Nong and Lam Dong); South East (Binh Phuoc, Tay Ninh, Binh Duong, Dong Nai, Ba Ria – Vung Tau and Ho Chi Minh City) and Mekong river delta (Long An, Tien Giang, Ben Tre, Tra Vinh, Vinh Long, Dong Thap, An Giang, Kien Giang, Can Tho, Hau Giang, Soc Trang, Bac Lieu and Ca Mau) (GSO 2010). Figure 2-1 shows the administrative map of Viet Nam (Source: Cartographic Publishing House, Vietnam).

2.2.2 Population and ethnicity

According to the result of the Viet Nam population and housing census, the Vietnamese population was 85,846,997 inhabitants in April 2009. Viet Nam is a multi-ethnic country with 54 ethnic groups, among which the Kinh ethnic group accounts for 86% of population. Most of the other ethnic groups live in the Northern Midlands and Mountain areas and the Central Highlands, in which they account for 55% and 35% of population, respectively. The Khmer ethnic group mainly live in the Mekong river delta (CP&HCSC 2010).

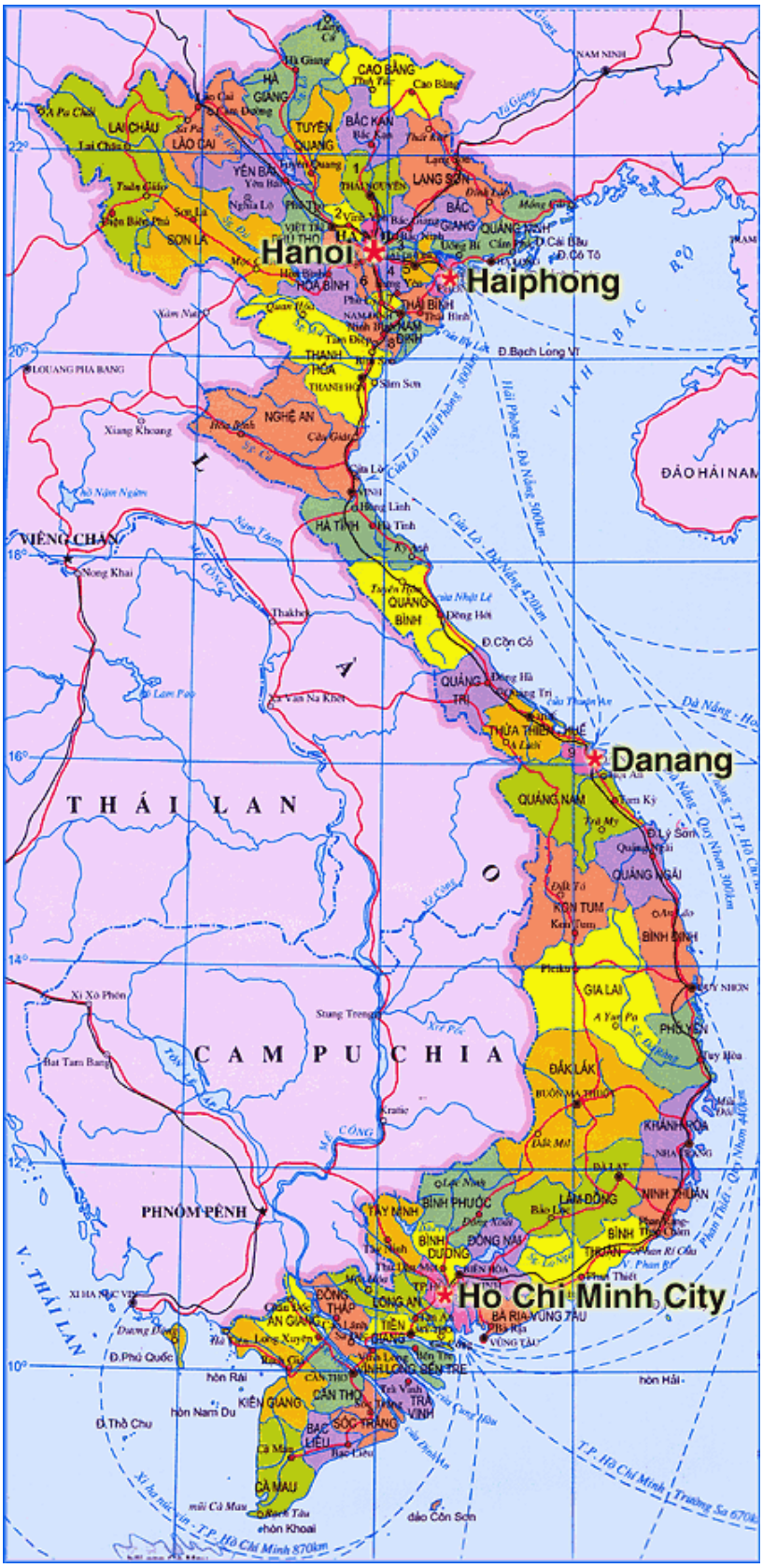
2.2.3 Health care system

The health care system of Viet Nam has been established at four levels, including commune, district, provincial and national levels. There are well developed and reasonably efficient referral patterns from commune health centre to district hospital, then to provincial hospital and finally to central hospital (national referral hospital). The commune health centre is the first place providing health care services

in the community, such as primary health care, first aid, immunizations, family planning, and detection and reporting of outbreaks to the higher level.

There were 1002 hospitals, 682 regional polyclinics and 10979 commune health centres with 27.1 beds and 6 physicians per 10,000 population, according to the Statistical Yearbook of Viet Nam 2009 and World Health Statistics 2010 (GSO 2010; WHO 2010). Total annual expenditure on health was 76 USD per capita and accounted for 7.3% of the Gross Domestic Product (GDP) in 2008. Life expectancy at birth was 73 years and neonatal, infant and under-five mortality rates were 9, 12 and 14 per 1,000 live births (WHO 2010).

Figure 2-1 Administrative map of Viet Nam



2.2.4 Hospital for Tropical Diseases

The Hospital for Tropical Diseases (HTD) in Ho Chi Minh City, previously called Cho Quan hospital, is a tertiary referral hospital for infectious diseases in the south of Viet Nam. It has 550 beds, 14 clinical wards and a laboratory centre for haematology, biochemistry, microbiology, virology, parasitology and mycology. This hospital has a catchment population of 40,000,000 people and receives approximately 35,000 inpatients every year. The Viet-Anh ward, which has 17 beds with a dedicated 5 beds intensive care unit, receives all patients with central nervous system (CNS) infections and severe malaria patients, except those with known HIV/AIDS.

2.2.5 Oxford University Clinical Research Unit Viet Nam

The Oxford University Clinical Research Unit Viet Nam (OUCRU-VN) was established at the Hospital for Tropical Diseases (HTD) in 1991 in a collaboration between Oxford University and this hospital and supported by the Wellcome Trust. Its clinical and scientific research programme focuses on the most significant infectious diseases in Viet Nam, such as dengue infection, malaria, tuberculosis, enteric, CNS infection and animal health and zoonoses. OUCRU-VN has developed strong links not only with HTD, but also with other hospitals in Ho Chi Minh City, Ha Noi and other regions in Viet Nam.

2.3 Methods

2.3.1 A prospective descriptive surveillance study of CNS infections (01SS study)

2.3.1.1 Study design and setting

This study was designed as a prospective hospital-based descriptive surveillance study to describe the aetiology and epidemiology of CNS infections in southern and central Vietnam. The majority of patients with central nervous system infections are admitted to provincial hospitals, which functions as the tertiary referral hospital in each province, because it is a serious and life-threatening disease. A lumbar puncture procedure is rarely done at a district hospital. Hence, I decided to recruit patients at provincial hospitals (see Figure 2-2). I selected provinces at a distance of at least 150 km from Ho Chi Minh City (HCMC) to determine incidence rates for provinces, since patients from provinces close to HCMC may be admitted directly to the hospitals in this city, potentially resulting in underestimation of provincial incidence rates. In the Mekong river delta, I chose 7 provincial hospitals (Dong Thap, An Giang, Kien Giang, Soc Trang, Bac Lieu, Ca Mau, Tra Vinh), 1 central hospital (Can Tho) and 1 district hospital (Sa Dec). Can Tho Central hospital, the biggest hospital in the Mekong river delta, is the tertiary referral hospital of Can Tho city. Sa Dec, the former administrative center of Dong Thap province before 1994, is the second city of this province. A substantial number of patients who live in districts at the south bank of Tien river usually attend to Sa Dec district hospital. Therefore, Sa Dec district hospital was also included, in addition to Dong Thap provincial hospital. I also chose Binh Phuoc provincial hospital in the South East, Dak Lak provincial hospital in the Central Highlands, Khanh Hoa provincial hospital in

the Central coastal areas and Hue Central hospital in the North Central of Viet Nam. I designated the Central coastal areas and North Central of Viet Nam as Central Viet Nam.

The average population and age structure of the population of these 12 provinces are presented in Table 2-1 and Table 2-2 (GSO 2009; CP&HCSC 2010; GSO 2010).

Table 2-1 Average population of surveillance provinces in two years 2008-2009

Province	Average population (× 10^5 inhabitants)	
	2008	2009
Ca Mau	12.017	12.070
Bac Lieu	8.475	8.584
Soc Trang	12.851	12.932
Can Tho	11.809	11.896
Tra Vinh	10.008	10.044
Dong Thap	16.625	16.677
An Giang	21.426	21.492
Kien Giang	16.723	16.879
Binh Phuoc	8.580	8.775
Dak Lak	17.151	17.331
Khanh Hoa	11.493	11.597
Thua Thien – Hue	10.849	10.887

Table 2-2 Population of surveillance provinces by age group in April 2009

Province	Population (× 10^5 inhabitants), n (%)						Total
	< 5	5-14	15-29	30-44	45-59	≥ 60	
Ca Mau	0.96033 (7.96)	2.08880 (17.31)	3.77247 (31.26)	2.69800 (22.35)	1.73719 (14.39)	0.81259 (6.73)	12.06938 (100.00)
Bac Lieu	0.61349 (7.16)	1.41374 (16.51)	2.78211 (32.48)	1.89115 (22.08)	1.25600 (14.66)	0.60869 (7.11)	8.56518 (100.00)
Soc Trang	1.01367 (7.84)	2.15830 (16.69)	3.90859 (30.23)	2.94606 (22.79)	1.92498 (14.89)	0.97693 (7.56)	12.92853 (100.00)
Can Tho	0.88719 (7.47)	1.69437 (14.26)	3.65884 (30.79)	2.92971 (24.65)	1.79244 (15.08)	0.92180 (7.76)	11.88435 (100.00)
Tra Vinh	0.75551 (7.53)	1.56831 (15.64)	3.01903 (30.10)	2.29511 (22.88)	1.57996 (15.75)	0.81220 (8.10)	10.03012 (100.00)
Dong Thap	1.30388 (7.82)	2.71592 (16.30)	4.73338 (28.40)	4.07945 (24.48)	2.45731 (14.75)	1.37473 (8.25)	16.66467 (100.00)
An Giang	1.73988 (8.12)	3.46653 (16.18)	6.25032 (29.42)	5.27347 (24.82)	2.91764 (13.73)	1.77925 (8.37)	21.24709 (100.00)
Kien Giang	1.36284 (8.07)	2.96728 (17.58)	5.28025 (31.28)	3.90191 (23.11)	2.23246 (13.22)	1.13774 (6.74)	16.88248 (100.00)
Binh Phuoc	0.84521 (9.68)	1.70434 (19.51)	2.57899 (29.52)	1.98298 (22.70)	1.14401 (13.09)	0.48045 (5.50)	8.73598 (100.00)
Dak Lak	1.59609 (9.21)	3.83971 (22.15)	5.01705 (28.94)	3.76013 (21.69)	2.12665 (12.27)	0.99661 (5.75)	17.33624 (100.00)
Khanh Hoa	0.93743 (8.10)	2.05549 (17.76)	3.21538 (27.78)	2.83449 (24.48)	1.58783 (13.72)	0.94542 (8.17)	11.57604 (100.00)
Thua Thien - Hue	0.85206 (7.83)	2.14076 (19.69)	2.99984 (27.59)	2.27876 (20.95)	1.48647 (13.67)	1.11631 (10.27)	10.87420 (100.00)
Total	12.86758 (8.09)	27.81355 (17.50)	47.21625 (29.70)	36.87122 (23.19)	22.24294 (13.99)	11.96272 (7.52)	158.97426 (100.00)

Figure 2-2 Locations of study sites (red dots)

(Source of map: http://www.learnnc.org/lp/multimedia/3311)

The study started at 10 provincial hospitals, 2 central hospitals and 1 district hospital in succession from August 2007 to April 2010. The time frames for calculation of incidence rates of CNS infection for each of the provinces were chosen in one year period or in two years period according to period of recruitment (see Table 2-3).

Table 2-3 Periods of recruitment and time frames for calculation of incidence rates of CNS infections

Hospital	Participants	Period of recruitment	Time frame for calculating incident rate
Ca Mau	Adults	Aug 2007 – Mar 2009	Feb 2008 – Jan 2009
	Children	Feb 2008 – Mar 2009	
Bac Lieu[1]	Adults	Aug 2007 – Mar 2009	Feb 2008 – Jan 2009
	Children	Feb 2008 – Aug 2008	
Soc Trang	Adults	Aug 2007 – Apr 2010	Feb 2008 – Jan 2010
	Children	Feb 2008 – Apr 2010	
Can Tho	Adults	Sep 2009 – Aug 2010	Sep 2009 – Aug 2010
	Children	-	
Tra Vinh	Adults	Feb 2008 – Mar 2009	Feb 2008 – Jan 2009
	Children	Feb 2008 – Mar 2009	
Dong Thap	Adults	Dec 2007 – Mar 2010	
	Children	Dec 2007 – Mar 2010	
Sa Dec	Adults	Jan 2008 – Mar 2009	Feb 2008 – Jan 2009
	Children	Jan 2008 – Mar 2009	

An Giang	Adults	Mar 2008 – Mar 2010	Mar 2008 – Feb 2010
	Children	Mar 2008 – Mar 2010	
Kien Giang	Adults	Dec 2007 – Mar 2010	Mar 2008 – Feb 2010
	Children	Feb 2008 – Mar 2010	
Binh Phuoc	Adults	Jan 2008 – Mar 2009	Mar 2008 – Feb 2009
	Children	Jan 2008 – Mar 2009	
Dak Lak	Adults	Dec 2007 – Apr 2010	Jan 2008 – Dec 2009
	Children	Dec 2007 – Apr 2010	
Khanh Hoa	Adults	Oct 2007 – Apr 2010	Jan 2008 – Dec 2009
	Children	Oct 2007 – Apr 2010	
Hue	Adults	Mar 2008 – Apr 2010	Apr 2008 – Mar 2010
	Children	Mar 2008 – Apr 2010	

[1] Incidence rates were only calculated for adult patients because the period of recruitment in children group was less than 1 year.

2.3.1.2 Inclusion and exclusion criteria

2.3.1.2.1 Inclusion criteria

Patients who satisfied the clinical criteria of suspected central nervous system infection were included if they:

- were at least one month of age.
- had fever ≥ 38 ^{0}C axillary.
- had at least one of the following symptoms: headache, neck stiffness, altered consciousness, focal neurology signs.
- had a cerebrospinal fluid (CSF) sample taken.

2.3.1.2.2 Exclusion criteria

Patients were excluded if they did not provide written informed consent.

2.3.1.3 Recruitment

During the period of surveillance, all patients who fulfilled the inclusion criteria were included in the study. Informed consent was obtained or proxy consent from a relative was obtained for a patient with GCS score <15 or a patient under 15 year-old on admission. The attending physician examined the patient and filled in a standardized data form. Data forms included general demographics, base line data, past medical history of underlying diseases, pig exposures, clinical manifestations, laboratory findings, treatment and outcome.

2.3.1.4 Sample collection

Four millilitres of CSF were taken from each patient. Three millilitres CSF were sent to the hospital laboratory for cell count, biochemistry measurements and microbiology investigations according to standard care. One millilitre was stored in a freezer at – 20^0C on the ward or in the microbiology laboratory, and these CSF samples were transferred to HTD every two months on dry ice for further investigations, including 200µl for diagnostic PCR (bacteria and virus), 100µl for JE and Dengue serology, 200µl for "pathogen discovery" and 500µl stored in a freezer at – 80^0C. In addition, all the bacterial isolates from CSF and blood cultures were also sent to HTD to confirm bacterial identification and antimicrobial susceptibility.

2.3.1.5 Definitions

2.3.1.5.1 Adults and children

Patients were assigned as adults if they were older than or equal to 15 years of age on the day of admission. If they were less than 15 years of age, they were assigned as children.

2.3.1.5.2 Case definitions of clinical syndromes

Clinical syndromes were defined in accordance with WHO case definitions of bacterial meningitis and Japanese encephalitis, and using a consensus case definition of tuberculous meningitis (WHO 2003; Marais et al. 2010), as described below.

2.3.1.5.2.1 Unsuspected (No) CNS infection

A patient was considered not to have a CNS infection in the presence of

- Normal CSF parameters AND
- No pathogen confirmed AND
- Discharge diagnosis of patient was stroke, epilepsy, mental disorders, drug/alcohol toxicity, hepatic encephalopathy, or sepsis, or benign febrile convulsion in children.

2.3.1.5.2.2 Bacterial meningitis

2.3.1.5.2.2.1 Laboratory-confirmed bacterial meningitis

Bacterial meningitis was considered laboratory-confirmed in the presence of at least one of the following criteria:

- Laboratory confirmation of bacterial infection by culture, Gram stain or real-time PCR methods of CSF.
- Positive bacterial blood culture and clinical syndrome consistent with bacterial meningitis (see below).

2.3.1.5.2.2.2 Probable bacterial meningitis

Probable bacterial meningitis was defined as the presence of the following criteria:

- Sudden onset of fever (>38^{0}C) (less than 7 days)
- AND at least one of the following signs:
  - Meningeal signs (neck stiffness, Kernig sign and Brudzinski sign)
  - Altered consciousness
- AND CSF examination showing at least one of the following:
  - Leukocytosis (≥ 10 cells/μl) AND at least 2 of the following criteria(*):
    - an elevated protein (> 1g/l)

- decreased glucose (<2.2 mmol/l or less than 50% of blood glucose)
- lactate ≥ 4 mmol/l

o Turbid appearance (when WC is missing or WC < 10/µl)

- AND no aetiologic agent was identified

(*) If lactate concentration was not available, a patient was diagnosed as BM when having at least one of the remaining criteria.

2.3.1.5.2.3 Viral encephalitis/meningitis

2.3.1.5.2.3.1 Laboratory-confirmed viral encephalitis/meningitis

Viral meningitis was considered laboratory confirmed in the presence of the following criteria:

o Laboratory confirmation by real-time PCR (enterovirus and *Herpes simplex*) or MAC ELISA (Japanese Encephalitis virus) on CSF.

o A case was classified as probable Japanese encephalitis (JE) if a patient had the following criteria:

- Fulfilled the case definition of probable viral encephalitis/meningitis (see below)
- JEV specific IgM titre in CSF was in the range from 8-12 U
- No other pathogen detected.

- A case was classified as Dengue encephalitis/meningitis, if Dengue virus specific IgM titre in CSF was higher than 12 U.
- A case was classified as possible Dengue encephalitis/meningitis if patient had the following criteria:
  - Fulfilled case definition of probable viral encephalitis/ meningitis (see below)
  - Dengue virus specific IgM titre in CSF was in the range from 8-12 U
  - No other pathogen detected.

2.3.1.5.2.3.2 Probable viral encephalitis/meningitis

Probable viral encephalitis or meningitis was defined according to the following criteria:

- Acute onset of fever (less than 7 days)
- AND at least one of the following:
  - Meningeal signs (neck stiffness, Kernig sign, and Brudzinski sign)
  - Change in mental status (confusion, disorientation, coma or inability to talk)
  - New onset of seizures (excluding simple febrile seizures)
- AND CSF examination showing at least one of the following:
  - Leukocytosis (≥10 cells/μl) AND at least 2 of these criteria

- protein ≤ 1g/l
- normal glucose (≥ 2.2 mmol/l or ≥ 50% of blood glucose)
- lactate < 4 mmol/l (*)

o Clear appearance (when WC is missing or WC < 10/µl)

- AND no aetiologic agent was identified

(*) *If lactate concentration was not available, a patient was diagnosed as viral encephalitis/meningitis when having the two remaining criteria.*

2.3.1.5.2.4 Tuberculous meningitis

2.3.1.5.2.4.1 Laboratory-confirmed tuberculous meningitis

Laboratory confirmation by positive smear (acid-fast bacilli, AFB) or real-time PCR of CSF

2.3.1.5.2.4.2 Probable and possible tuberculous meningitis:

Probable and possible tuberculous meningitis were defined using the diagnostic criteria for classification of probable and possible tuberculous meningitis (Marais et al. 2010). The classification is depicted below (Table 2-4).

Table 2-4 Diagnostic score of probable and possible tuberculous meningitis

	Diagnostic score
Clinical criteria	(Maximum category score=6)
- Symptom duration of more than 5 days	4
- Systemic symptoms suggestive of tuberculosis (one or more of the following): weight loss (or poor weight gain in children), night sweats, or persistent cough for more than 2 weeks	2
- History of recent (within past year) close contact with an individual with pulmonary tuberculosis or a positive tuberculin skin test (TST) or interferon-gamma release assay (IGRA) (only in children <10 years of age)	2
- Focal neurological deficit (excluding cranial nerve palsies)	1
- Cranial nerve palsy	1
- Altered consciousness	1
CSF criteria	(Maximum category score=4)
- Clear appearance	1
- Cells: 10-500 per μl	1
- Lymphocytic predominance (>50%)	1
- Protein concentration greater than 1g/L	1
- CSF to plasma glucose ratio of less than 50% or an absolute CSF glucose less than 2.2 mmol/L	1

Cerebral imaging criteria	(Maximum category score=6)
- Hydrocephalus	1
- Basal meningeal enhancement	2
- Tuberculoma	2
- Infarct	1
- Pre-contrast basal hyperdensity	2
Evidence of tuberculosis elsewhere	(Maximum category score=4)
- Chest radiograph suggestive of active tuberculosis: signs of tuberculosis= 2; miliary tuberculosis= 4	2/4
- CT/ MRI/ ultrasound evidence for tuberculosis outside the CNS	2
- AFB identified or *Mycobacterium tuberculosis* cultured from another source, such as sputum, lymph node, gastric washing, urine, blood culture	4
- Positive commercial *M. tuberculosis* nucleic acid amplification test (NAAT) from extra-neural specimen	4

Probable tuberculous meningitis:

- Total diagnostic score of 10 or more points (when cerebral imaging is not available) or 12 or more points (when cerebral imaging is available)
- At least 2 points should either come from CSF or cerebral imaging criteria.

Possible tuberculous meningitis:

- Total diagnostic score of 6-9 points (when cerebral imaging is not available) or 6-11 points (when cerebral imaging is available)
- Possible tuberculosis cannot be diagnosed or excluded without doing a lumbar puncture or cerebral imaging.

2.3.1.5.2.5 Cryptococcal meningitis

An India ink stain of CSF positive with yeasts, or *Cryptococcus neoformans* isolated from CSF.

2.3.1.5.2.6 Eosinophilic meningitis

2.3.1.5.2.6.1 Confirmed eosinophilic meningitis

Eosinophilic meningitis was diagnosed in patients with meningitis and a percentage of eosinophils in CSF greater than 10% (Lo Re et al. 2003)

2.3.1.5.2.6.2 Probable eosinophilic meningitis

Probable eosinophilic meningitis was diagnosed if:

the percentage of eosinophilic cells in blood > 10%,

AND meningitis manifestations

AND no pathogen confirmed in CSF by culture, PCR or ELISA method.

2.3.1.5.3 Outcome of CNS infection

Outcomes of CNS infection, such as alive, death, transfer, and unknown outcome, at the day of discharge, was based on discharge data recorded by the attending physician at the provincial hospital.

2.3.1.6 Classification of central nervous system infection cases

We used the case definitions described to classify CNS infection cases in the following 4 steps.

- **Step 1:** Apply criteria of laboratory-confirmed bacterial meningitis and viral encephalitis/meningitis.
- **Step 2:** Exclude unsuspected CNS infection cases according to the criteria for unsuspected CNS infection.
- **Step 3:** Apply the consensus case definition of tuberculous meningitis (TBM) on the cases with CNS infection with unconfirmed pathogen.
    - In the group of patients with TBM score <6 points, we applied the case definition of probable bacterial meningitis (BM) and viral encephalitis/meningitis to classify cases as BM or viral encephalitis/meningitis.
    - In the group of patients with TBM score ≥6 points (possible TBM according to the diagnostic algorithm), we assigned a case as TBM if the patient was treated with anti-tuberculous drugs, died, was transferred to another hospital, or had an unknown outcome. If these patients recovered without TB treatment, we applied the case definition of probable bacterial meningitis (BM) and viral encephalitis/meningitis to classify a case as BM or viral encephalitis/meningitis.
- **Step 4:** *Mycobacterium tuberculosis* real-time PCR was done on all CSF samples of probable and possible TBM cases, i.e. all patients with TBM score ≥6 points assigned as TBM, to confirm the diagnosis.

2.3.1.7 HIV status

In Viet Nam, HIV test is not routinely done in all CNS infection patients. Patients are tested for HIV according to the decision of the attending physician relying on their risk of infection, such as intravenous drug abusers, sexual workers or unprotected sexual exposures. Patients are also tested HIV when they have cryptococcal or tuberculous meningitis.

2.3.1.8 Sample size

All patients who met the inclusion criteria and who were admitted to the participating provincial hospitals during the period of surveillance were recruited into the study (Table 2-3).

2.3.1.9 Statistical methods

All variables of interest were summarized by group (adult or children). Categorical variables were summarized as number and percent (%). Continuous variables were summarized as median and interquartile range (IQR). Baseline variables were compared between adult and children groups and between groups with bacterial and viral CNS infection, using Chi-square test or Fisher's exact test (when one or more of the expected count is less than 5) for categorical variables and Wilcoxon rank sum test for continuous variables. Logistic regression was used to assess univariate association of the pathogen of interest with age. In the analysis of aetiology of CNS infection, I excluded unsuspected CNS infection cases (see Section 2.3.1.4.2.1). I also excluded dual infection cases when analyzing pathogens of bacterial meningitis or viral encephalitis/meningitis separately. Seasonality of admission numbers of children and adults with CNS infections as well as that of *S.*

suis meningitis cases was assessed using Poisson regression models, which modelled the monthly admission counts depending on a hospital effect, a global linear trend and a sinusoidal shape for the seasonality effect (modelled with a sine- and a cosine-term, i.e. a cosinor model) (Barnett et al. 2010). The log-number of days per month was used as an offset in the model to correct for unequal lengths of the month. For testing seasonality I tested whether the sine- and the cosine-term could jointly be omitted from the model using a likelihood ratio test. Incidence rates of CNS infection were calculated according to patient's residence in one year of surveillance (Ca Mau, Bac Lieu, Can Tho, Tra Vinh, Dong Thap and Binh Phuoc) or in two years of surveillance (An Giang, Kien Giang, Soc Trang, Dak Lak, Khanh Hoa and Thua Thien – Hue). In Can Tho and Bac Lieu, I only calculated the incidence rate in adult population because we only recruited adult patients in Can Tho and the recruitment of paediatric patients in Bac Lieu only lasted 7 months. Average population of a year was calculated with assumption that the population changed regularly (GSO 2009; CP&HCSC 2010; GSO 2010).

$$\bar{S} = \frac{S1 + S2}{2}$$

$\bar{S}$: Average population

$S1$: Population at the beginning of year

$S2$: Population at the end of year.

I used data related to age group population of the Vietnam population and house census in April 2009 to estimate the person-time of observation of adult and children population (Table 2-2). The 95% confidence intervals of incidence rates were calculated as exact Poisson confidence intervals. Incidence rate in a province/region/age group was compared with other province/region/age group by

Poisson regression. The association between the number of *S. suis* cases and the pig density was tested with Poisson regression with the number of person years of follow-up in each province included as an offset. I used quasi-likelihood to adjust for over-dispersion. All analyses were performed with Stata version 10.1 (StataCorp) and R version 2.11.1 software.

2.3.1.10 Ethical approval

This study was approved by the Scientific and Ethics Committee of each study site (provincial hospitals), the Hospital for Tropical Diseases and the University of Oxford Tropical Research Ethics Committee (OXTREC 01-08).

2.3.2 A Case-control study to identify risk factors for *Streptococcus suis* infection in Vietnam (EN study)

2.3.2.1 Study design and setting

This study was designed as a case-control study, including one group of patients with invasive *S. suis* infection and two control groups: an unmatched hospital control group and a community control group, matched by residency and age. The ratio between cases and each control group was 1:3. The study was conducted at the Hospital for Tropical Diseases (HTD) between May 2006 and June 2009. The recruitment of cases and hospital control groups took place at the dedicated Central Nervous System (CNS) infectious disease ward (Viet-Anh ward) at HTD. Community controls were recruited according to the residency of the cases in the south of Viet Nam, mainly in Ho Chi Minh City and the provinces of the Mekong River Delta.

2.3.2.2 Inclusion and exclusion criteria

2.3.2.2.1 Inclusion criteria

Inclusion criteria are presented in Table 2-5.

Table 2-5 Inclusion criteria of EN study

Cases: - At least 15 year-old - *Streptococcus suis* meningitis or sepsis confirmed by blood culture or CSF culture or CSF real-time PCR positive - Admitted to CNS disease ward of HTD **Hospital controls:** - At least 15 year-old - Confirmed bacterial meningitis (not *S. suis*), eosinophilic meningitis, cryptococcal meningitis, viral encephalitis/meningitis or malaria (confirmed by blood smear) - Admitted to CNS disease ward of HTD **Community controls:** - Living in the same commune as case for at least 4 weeks until inclusion of case - Age matched with case (10 years range)

2.3.2.2.2 Exclusion criteria

Exclusion criteria are presented in Table 2-6.

Table 2-6 Exclusion criteria of EN study

- Did not provide informed consent.
- Recent history of bacterial meningitis (< 1 year before admission).
- Did not regain full consciousness (GCS 15) within 14 days after admission.
- Transferred to other hospitals shortly after admission (less than 7 days)
- HIV positive.

2.3.2.3 Recruitment

All consecutive patients admitted with signs and symptoms consistent with CNS infectious diseases, who met the inclusion criteria for case or hospital control, were recruited to the study (Table 2-5 and Table 2-6). If the patients fulfilled the inclusion criteria for a case or for a hospital control, informed consent was obtained. After inclusion of a patient as a case, the next three consecutive patients admitted to the ward who met the inclusion criteria were included as hospital controls. Three community controls, matched for age (within a 10 year age range), were randomly identified from a list of households available at the health centre in the community of

residence of the case, by using random number tables. We contacted with health workers at these centres, where the cases lived, to enroll community controls. Household members were identified either at the HTD, when taking care of a case or hospital control, or during the household visits. A maximum of three adult household members of each case and control were recruited to study if they agreed to take part in it.

2.3.2.4 CSF sample collection

As part of the standard clinical assessment on admission, a cerebrospinal fluid (CSF) sample was directly taken from each patient suspected of CNS infection at the Viet-Anh ward of the Hospital for Tropical Diseases (HTD). Three millilitres CSF was sent to the hospital laboratory for cell count, biochemistry measurements and microbiology investigations. One millilitre was stored in a freezer at -20^0C and was separately sent to the molecular diagnostic laboratory on the next day for diagnostic PCR for detection of *S. suis* serotype 2, *S. pneumoniae*, *H. influenzae* type b and *N. meningitidis*.

2.3.2.5 Collecting throat and rectal swab samples/stool samples of cases, controls and their household members

All patients admitted to the Viet-Anh ward of the HTD had a throat and rectum swab taken on admission, to detect carriage of *S. suis*. Fourteen days after admission or at discharge, a second throat and rectum sample were taken from patients recruited in the study. In a previous study on human *S. suis* meningitis at HTD, over 60% of patients received antimicrobial treatment prior to admission (Mai et al. 2008). As detection of potential carriage in cases and hospital controls could be

affected by their antimicrobial treatment, household members were also studied for carriage of *S. suis* serotype 2 since if carriage and associated transmission were to occur, household members of carriers are the most likely to become positive. Individuals were fully informed about the nature of the study prior to being asked for written informed consent. The neighbourhood controls and a maximum of three adult household members of each case and control included were invited to submit a throat sample and rectal swab or stool sample (whichever the individual preferred) for detection of carriage of *S. suis* serotype 2, at patient's admission or the first visit to the household and at 10 – 16 days after (Figure 2-3 and 2-4). We recorded sex, age, and occupation of household members

2.3.2.6 Definitions of potential risk factors under investigation

2.3.2.6.1 Occupational exposures: at least one of the following occupations

- Butcher
- Pig breeder
- Slaughterer
- Meat transporter
- Meat processing
- Veterinarian
- Cook

2.3.2.6.2 Contact with pigs/pork: at least one of the following contacts or activities

- Bathe pigs
- Feed pigs
- Clean up the piggery
- Slaughter pigs
- Prepare or handle blood, organs from pigs
- Visit a pig farm in the last 2 weeks

2.3.2.6.3 "High risk" dishes

- Pig/duck fresh blood
- Pig tonsils/tongue
- Pig stomach/intestines
- Pigs uterus
- Under-cooked pig blood

2.3.2.6.4 Skin injuries

Patients were checked by nurses and doctors for skin injuries on forearms, hands and feet. Injuries were defined as lesions with signs of disruption of skin integrity.

2.3.2.6.5 Underlying diseases

The presence of at least one of these conditions: alcoholism, diabetes mellitus and splenectomy.

2.3.2.6.6 Alcoholism

A person drinking beer >1500 ml/day or wine >250 ml/day in at least 5 days/week (Wine=SPIRIT 30-40^0)

2.3.2.6.7 Household exposure to pigs

Living in a household with any number of pigs around the house which belonged to the household.

2.3.2.6.8 Household members

An adult household member was defined as any adult (at least 15 years old) who resided for at least 50% of the week in the same house as the case or control.

2.3.2.7 Assessment of risk factors

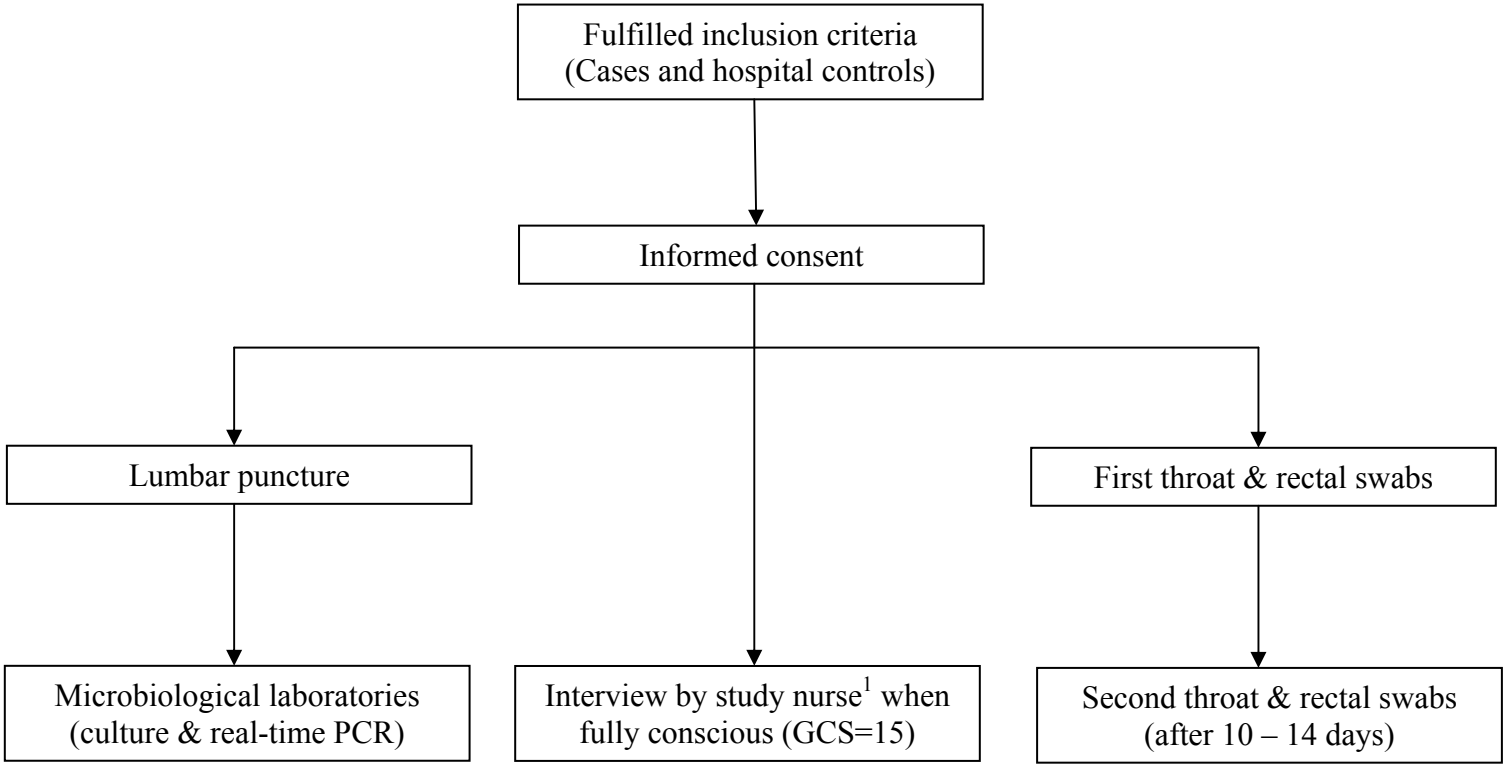
Risk factors were assessed from data collected using a standardized questionnaire. This questionnaire was developed in Vietnamese and validated at HTD and consisted of four parts: socio-demographic and cultural factors, medical history, potential exposure to pigs or pork and culinary habits and hygiene measures. The majority of the questions were “closed questions” but “open questions” which allowed participants to explain in their own words were also included. Patients and hospital controls were interviewed when they were fully conscious. The questionnaire was filled in by structured interview, which was carried out by one of two research nurses on the ward for cases and hospital controls, or at the residency of community controls. The interviewers were blinded towards the diagnosis of the patient in the case and hospital control groups. We focused on potential risk factors identified from

the literature, including underlying diseases (alcoholism, diabetes mellitus and splenectomy), occupational exposures, pigs or pork exposures, skin lesions, and household exposures to pigs. In addition, we hypothesized that consumption of "high risk" food dishes, potentially contaminated with *S. suis*, could function as a source of infection.

2.3.2.8 Human carriage of *Streptococcus suis*

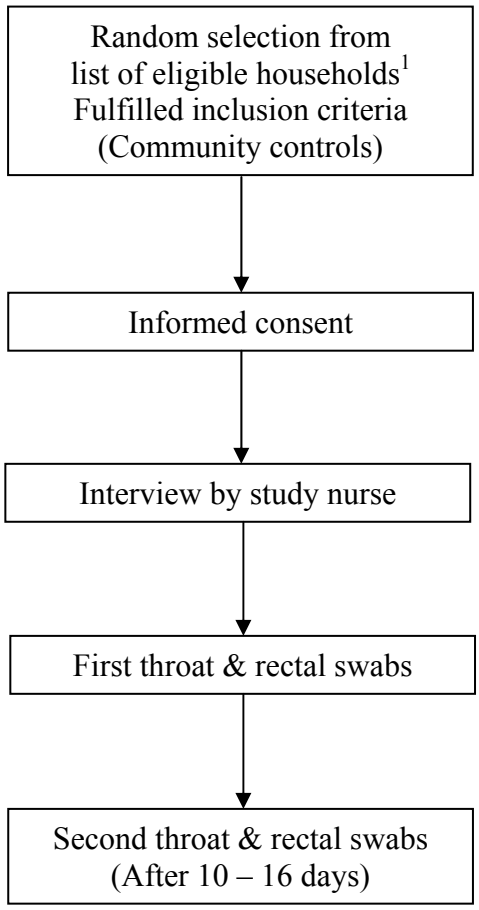
Participants were assigned as confirmed carrier if they had 2 PCR positive throat or rectal swab samples taken on 2 separate occasions with a minimum of 14 days in between. Possible carriage was defined as a single PCR positive throat or rectum swab sample/stool sample or a positive throat and rectum sample taken on the same day.

Figure 2-3 Recruitment of cases and hospital controls



[1] Study nurses were unaware of case or control status of patients

Figure 2-4 Recruitment of community controls



[1] Eligible households were defined as households in the same commune as a case

2.3.2.9 Detection of tonsillar carriage of *S. suis* serotype 2 in pigs in the households of cases and controls

For all cases and controls who reported exposure to pigs at home, sampling of the pigs was performed in collaboration with the Sub-department of Animal Health of Ho Chi Minh City. Pigs were sampled for all cases and controls who reported exposure to pigs at home. Swab samples were taken from all pigs present (except pregnant sows to avoid stress-induced miscarriage) within 4 weeks of admission of the patient, or at the second visit to the community controls. Pigs were kept by a household member or by a special lasso. To avoid contamination of the swab sample with the bacteria in pig mouth, the veterinarian, who took the swab sample, used a rope to keep the mandible and tongue of pig out of the way when taking tonsil swab sample (Figure 2-5 and 2-6).

Figure 2-5 Sterile cotton inoculating swab

Figure 2-6 Taking pig swab samples

2.3.2.10 Sample size

There was no data available from previous studies that allowed a definitive sample size calculation. However, when retrospectively looking at demographic data obtained during a randomized study on the efficacy of adjunct dexamethasone in the treatment of acute bacterial meningitis, 26% (59/226) of the non-*S. suis* patients (all other patients combined with different or unknown causes of meningitis) had potential

occupational exposure to pigs (e.g. farmer, butcher) compared with 59% (46/78) of *S. suis* meningitis patients (Nguyen et al. 2007), corresponding to an odds ratio of approximately 4. We decided on a target sample size of 100 cases (and 300 matched controls), corresponding to a recruitment period of approximately 4 years and a target odds ratio for 80% power of 2.1 assuming a probability of exposure in controls of 0.25 and a correlation coefficient for exposure between matched cases and controls of at most 0.2. With 100 cases, we also expected to fit reliable multivariate models with up to 10 covariates without over fitting the data (Harrell 2001).

2.3.2.11 Statistical methods

All variables of interest were summarized by group (case, hospital, community control and household member). Categorical variables were summarized as number and percent (%). Continuous variables were summarized as median and interquartile range (IQR). To assess univariate associations of *S. suis* with potential risk factors in hospital controls, we used both logistic regression without any adjustment for covariates and with adjustment for sex, age, and living in a rural or urban area. We used conditional logistic regression for the matched community controls and these analyses were performed with and without additional adjustment for sex (in addition to the matched variables age and place of living). In a multivariate analysis of potential risk factors of main interest, all potential risk factors plus the potential confounders (e.g. age, sex, place of living) were jointly included in a logistic (hospital controls) or conditional logistic (community controls) regression model. No model selection such as backwards elimination was performed. In the medical literature, *S. suis* infection through minor cuts or abrasions of the skin has been reported in individuals having contact with pigs or pork (Arends et al. 1988; Yu et al. 2006).

Therefore, we included separate effects of exposure to pigs or pork depending on whether the individual had skin injuries or not. *S. suis* infection occurred predominantly in males but controls were not matched by gender. As a sensitivity analysis, we therefore repeated the multivariate analysis including only male cases and controls. All analyses were performed with Stata version 10.1 (StataCorp) software.

2.3.2.12 Ethical approval

This study was approved by the Scientific and Ethics Committee of the Hospital for Tropical Diseases and the University of Oxford Tropical Research Ethics Committee (OXTREC 012-06).

2.4 Microbiological methods

2.4.1 Gram stain of cerebrospinal fluid sample

2.4.1.1 Prepare a slide smear

- Aseptically collected specimen of CSF was sent to the bacteriology laboratory as soon as possible after collection. The CSF was then aseptically decanted into sterile centrifuge tubes and centrifuged at 4000 rpm for 4 minutes.

- Transfer a drop of deposit to be examined on a slide with an inoculation loop and spread the sample with inoculation loop to an even thin film over a circle of 1.5 cm in diameter. On each slide, only one clinical specimen was applied.

2.4.1.2 Gram staining

Gram staining was carried out in 4 steps:

- Step1: Staining with crystal violet solution for 60 seconds and rinsing.

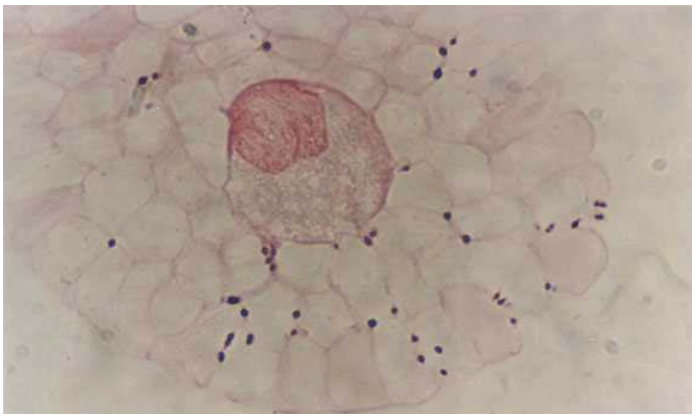
- Step 2: Staining with the iodine solution for 30 seconds and rinsing.

- Step 3: Decolouring by ethanol/acetone for 5 seconds and rinsing.

- Step 4: Counterstain with safranin solution for 20 seconds and rinsing.

Finally, the slide is observed under a microscope (Figure 2-7)

Figure 2-7 Gram positive cocci on Gram stain of CSF sample



2.4.2 Blood and cerebrospinal fluid (CSF) culture:

2.4.2.1 Blood and CSF culture at the Hospital for Tropical Diseases (HTD)

2.4.2.1.1 Blood culture

Blood samples were taken on admission for all patients and blood cultures were performed in the microbiology laboratory of the HTD using the BD BACTEC® 9050 blood culture system, using standard culture and identification methods.

2.4.2.1.2 Cerebrospinal fluid (CSF) culture

- Aseptically collected specimen of CSF is sent to the bacteriology laboratory as soon as possible after collection.
- The CSF is then aseptically decanted into sterile tubes and centrifuged at 4000 rpm for 4 minutes.
- The deposit is cultured on blood agar, chocolate agar and in brain heart infusion broth using sterile bacteriological loops.

- These cultures are incubated overnight at 35 ^{0}C in 5% CO_2 for 16 – 18 hours and then examined for growth.

- Any colonies growing are identified by Gram stain and full identification carried out along with antibiograms.

2.4.2.2 Blood and CSF culture at provincial hospitals

Blood and CSF cultures were performed in the microbiology laboratory of provincial hospitals using standard culture methods. Blood culture was performed by the BD BACTEC® 9050 blood culture system (BD Microbiology, USA) in Dong Thap and Kien Giang hospitals, and by BacT/Alert blood culture system (bioMérieux, France) in Hue Central hospital. Manual blood culture using commercially available blood culture media made in Vietnam were used in the remaining hospitals. The CSF cultures were incubated overnight in candle jars, except in Hue Central hospital where a CO2 incubator was available. Laboratories used the locally available tools for identification of isolates from positive cultures. In addition, all bacterial isolates were sent to HTD for bacterial identification and antimicrobial susceptibility testing.

2.4.3 Culture of throat and rectal swab samples of humans and pigs

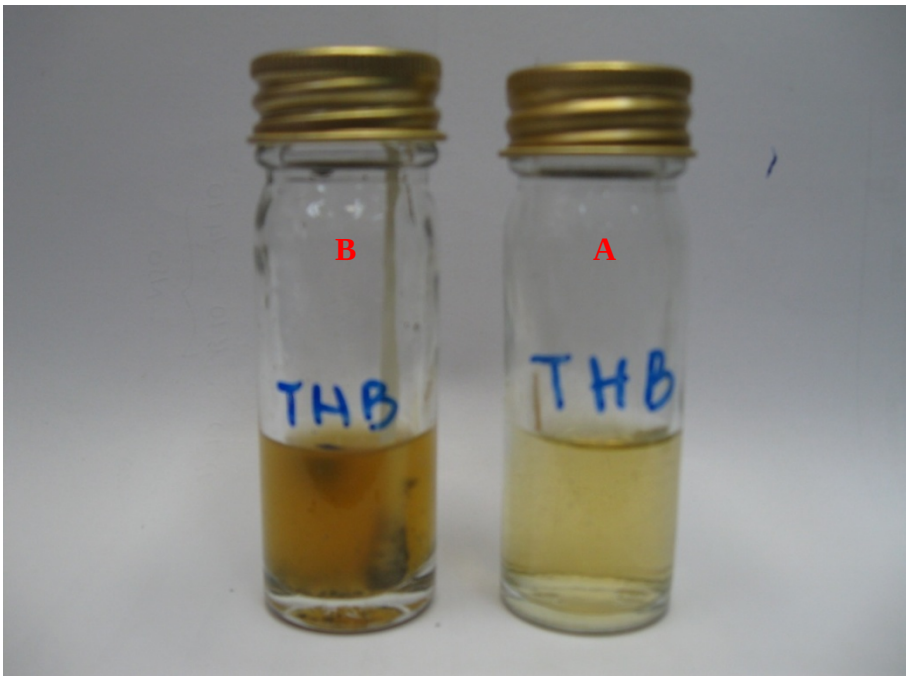
2.4.3.1 Culture of human swab samples

Swab samples were inoculated in transport medium (TRANSWABS®, UK) at the site, and transferred to the laboratory at HTD or stored at 4^{0}C until transfer within 48 hours (Figure 2-8).

Figure 2-8 Swab samples were inoculated in transport medium

At the laboratory, swabs were inoculated into selective Todd-Hewitt broth (OXOID, UK), containing Streptococcal Selective Reagent (Oxoid) and crystal violet (Wisselink et al. 1999) and incubated overnight at 37^0C, followed by real-time PCR for detection of *S. suis* serotype 2 (Figure 2-9). Positive samples were cultured to retrieve *S. suis* isolates.

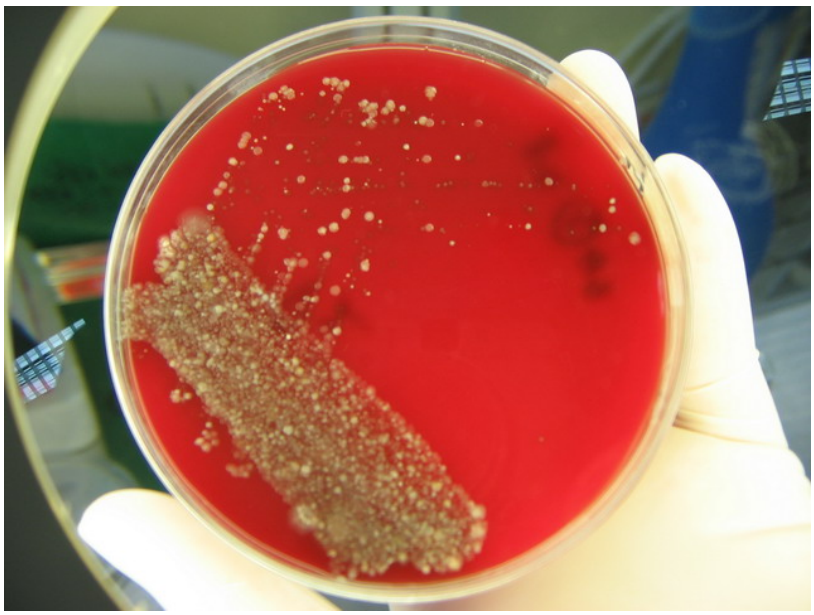
Figure 2-9 Selective Todd-Hewitt broth (OXOID, UK) [A] and after incubation overnight at 37^0C [B]



2.4.3.2 Culture of pig tonsils swab samples

Samples were cultured on blood agar (Figure 2-10) and were also inoculated into selective Todd-Hewitt broth (OXOID, UK). Both media contained Streptococcal Selective Reagent (Oxoid) and crystal violet (Wisselink et al. 1999). After that, they were incubated overnight at 37^0C, followed by real-time PCR for detection of *S. suis* serotype 2 of the Todd-Hewitt broth. Positive samples were cultured to retrieve *S. suis* isolates for further analysis.

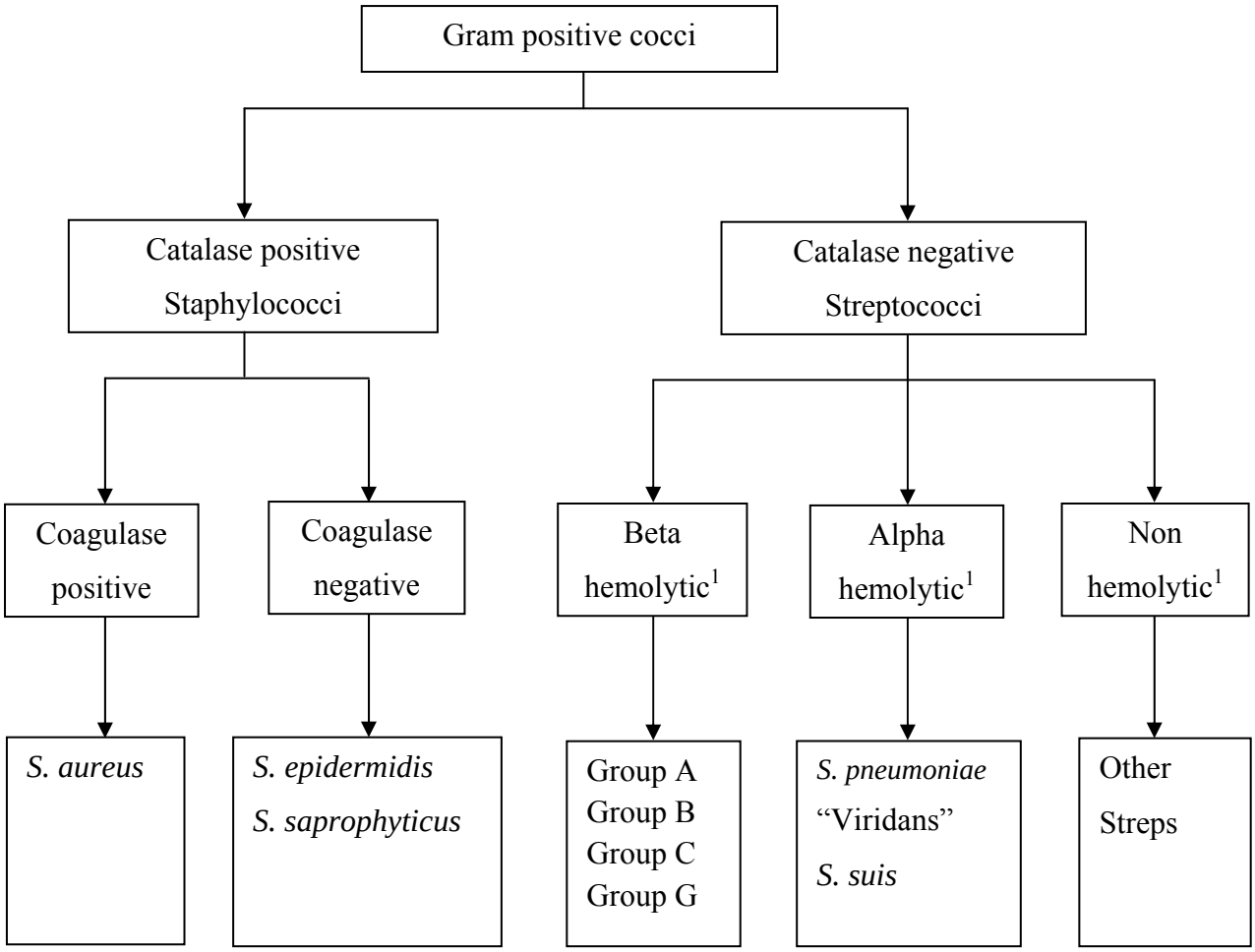
Figure 2-10 Pig tonsil swab sample was cultured on blood agar



2.4.4 Identification of bacterial strains

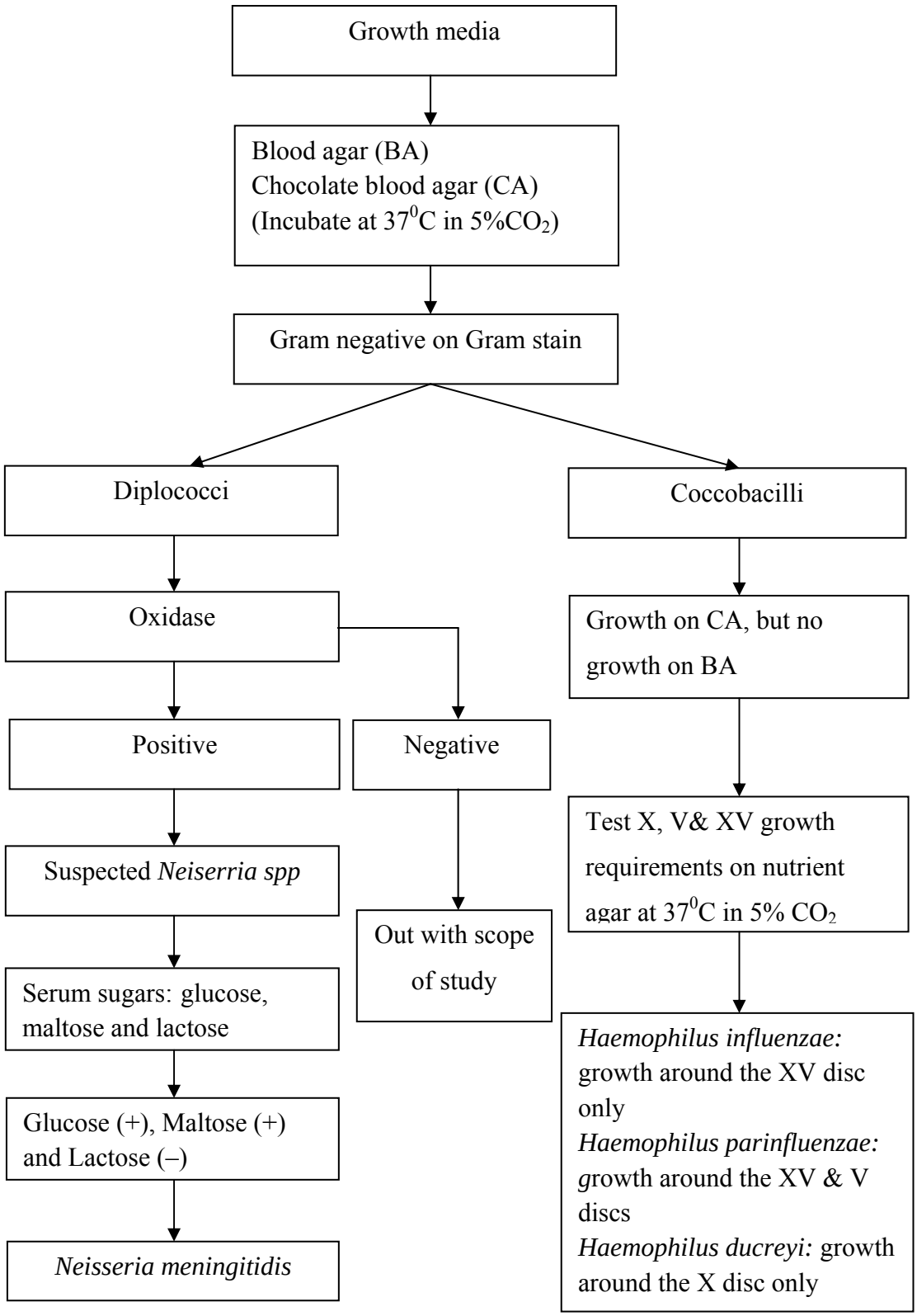
All of the bacterial strains isolated from CSF and/or blood culture were identified according to the following figures (Figure 2-9 and 2-10).

Figure 2-11 Identification of Gram positive cocci from CSF and blood culture



[1] on sheep blood agar plate

Figure 2-12 Identification of Gram negative organisms from CSF culture



2.4.5 *Streptococcus suis* identification and antimicrobial susceptibility

2.4.5.1 Identification of *Streptococcus suis* strains

S. suis was identified on the basis of colony morphology, negative catalase reaction, optochin resistance, and by APIStrep (Biomerieux, France) and subsequently serotyped by slide agglutination (Statens Serum Institute, Denmark) (Figure 2-13, 2-14 and 2-15).

Figure 2-13 *Streptococcus suis* colonies on blood agar

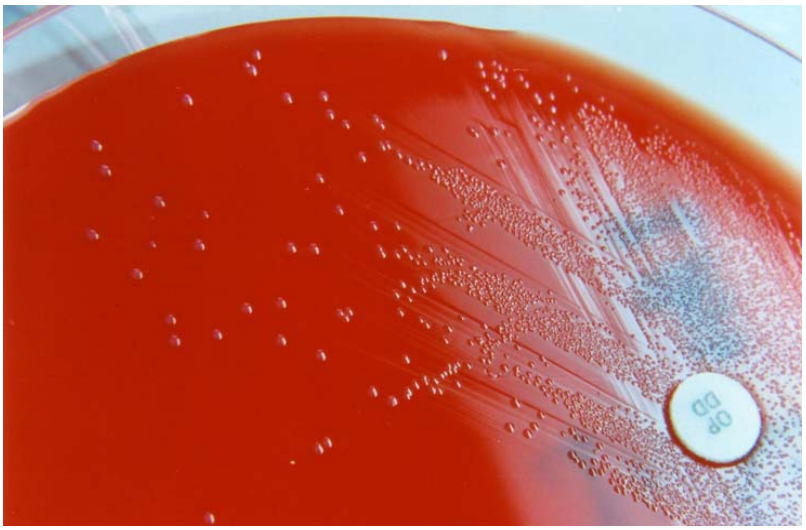


Figure 2-14 *Streptococcus suis* was identified by APIStrep (Biomerieux, France)

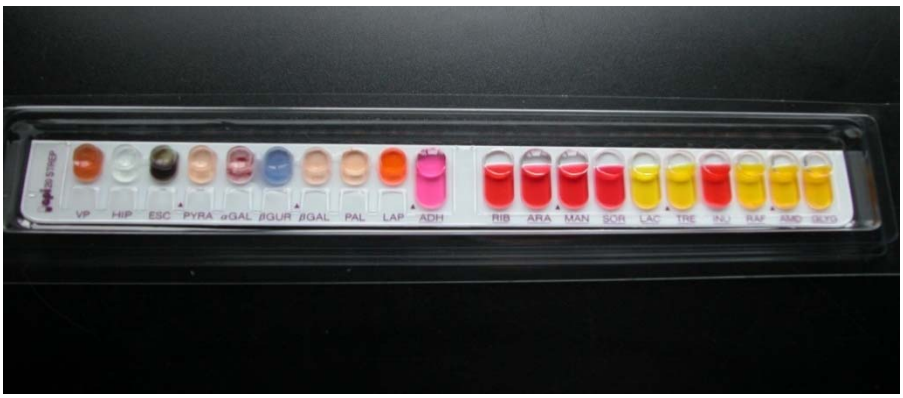


Figure 2-15 Identifying serotype of *Streptococcus suis*

2.4.5.2 Antimicrobial susceptibility of *Streptococcus suis* isolates

Antimicrobial susceptibility of *Streptococcus suis* isolates was determined with E-test (AB Biodisk, Sweden), and results were interpreted according to Clinical and Laboratory Standards Institute criteria (Figure 2-16 and Table 2-7) (CLSI 2010) using breakpoints defined for *Streptoccus* species, viridans group.

Figure 2-16 Antimicrobial susceptibility was determined by E-test (AB Biodisk, Sweden)

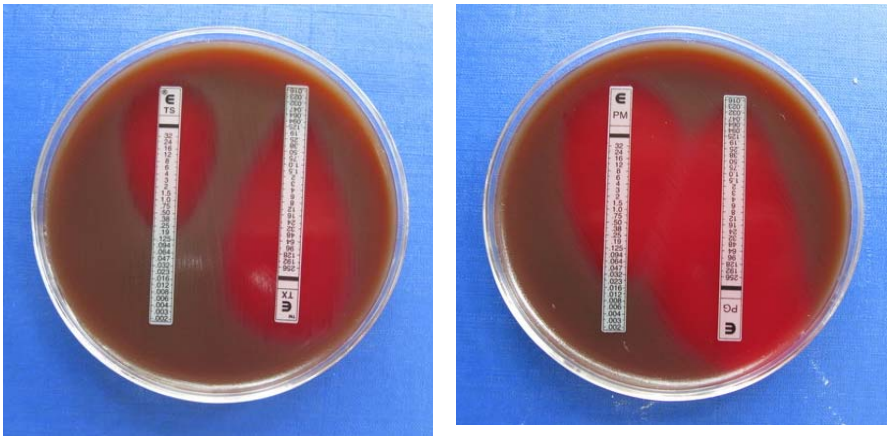


Table 2-7 Zone diameter interpretive standards and equivalent minimal inhibitory concentration (MIC) breakpoints for *Streptococcus* spp (viridans group)

Antimicrobial agent	Disk content	Zone diameter (mm)			Equivalent MIC breakpoints (μl/ml)	
		R	I	S	R	S
Penicillin	10 units	N/A[1]	N/A[1]	N/A[1]	≥ 4	≤ 0.12
Ceftriaxone	30 μg	≤ 24	25-26	≥ 27	≥ 4	≤ 1
Vancomycin	30 μg	N/A[1]	N/A[1]	≥ 17	N/A[1]	≤ 1
Levofloxacin	5 μg	≤ 13	14-16	≥ 17	≥ 8	≤ 2
Chloramphenicol	30 μg	≤ 17	18-20	≥ 21	≥ 16	≤ 4
Erythromycin	15 μg	≤ 15	16-20	≥ 21	≥ 1	≤ 0.25
Tetracycline	30 μg	≤ 18	19-22	≥ 23	≥ 8	≤ 2

[1] not available.

2.5 Molecular methods

In this work internally controlled real-time PCR was used for the detection of *S. pneumoniae*, *H. influenzae* type b, *N. meningitidis*, *S. suis* serotype 2, *Herpes simplex* 1/2, enteroviruses (generic) and *Mycobacterium tuberculosis* in CSF samples. The real-time PCR for detection of *S. suis* serotype 2 was also used on swab cultures.

DNA and RNA were extracted from CSF or swab samples, which had already been pre-lysed with protein denaturants, using the EasyMag extraction system (BioMerieux, Ho Chi Minh City, Vietnam), according to manufacturer's instructions. The DNA and RNA extractions were then subjected to consecutive duplex real-time PCRs, of which specific primers and probes are listed in Table 2-9, which amplified a single specific DNA sequence of a pathogen of interest and an internal control DNA sequence. Except for *Mycobacterium tuberculosis* PCR, the internal control DNA sequence was *gB* gene of Phocid Herpes virus and the internal control RNA sequence was *NSP* gene of Equine arteritis virus (van Doornum et al. 2003; Scheltinga et al. 2005).

An internally controlled real-time PCR for the detection of *S. suis* serotype 2 in cerebrospinal fluid samples was developed which targeted the *cps2J* gene of *S. suis* serotype 2. Sensitivity and specificity of the assay in culture confirmed clinical samples were 100% (Nga et al. 2011). We applied RealAccurate^{TM} *Mycobacterium tuberculosis* PCR kit (Patho Finder, The Netherlands), targeting the IS6110 sequence, to confirm tuberculous meningitis. This PCR kit had been validated by a set of CSF samples, including 24 TBM CSF samples confirmed by CSF smear (AFB) or mycobacterial growth indicator tube (MGIT) culture method, 9 pneumococcal meningitis CSF samples, 3 meningococcal meningitis CSF samples, 2 *E. coli*

meningitis CSF samples, 2 *Listeria spp* meningitis CSF samples, 1 *Herpes simplex* encephalitis meningitis and 1 unconfirmed viral encephalitis CSF samples. The sensitivity, specificity, positive predictive value and negative predictive value of it were 66.67%, 94.44%, 94.12% and 68.00%, respectively (Table 2-8).

Table 2-8 Evaluation of RealAccurate™ PCR kit in TBM diagnosis

		MTB[1]		Total
		Yes	No	
Test result	**Positive**	16	1	17
	Negative	8	17	25
Total		24	18	32

[1] as confirmed by smear and/or culture

Sensitivity: 66.67 %

Specificity: 94.44 %

Positive predictive value: 94.12 %

Negative predictive value: 68.00 %

Table 2-9 Specific primers and probes used in this study

Pathogen	Gene target	Oligo sequence (5' – 3')			Reference
		Forward	Reverse	Probe	
Streptococcus pneumoniae	*Ply*	TGCAGAGCGTCCTTTG GTCTAT	CTCTTACTCGTGGTTTCC AACTTGA	FAM-TGGCGCCCATAAGCAACACT CGAA-TAMRA	(Corless et al. 2001)
Haemophilus influenzae type b	*bexA*	GGCGAAATGGTGCTGG TAA	GGCCAAGAGATACTCAT AGAACGTT	FAM-CACCACTCATCAAACGAATG AGCGTGG-TAMRA	(Corless et al. 2001)
Neisseria meningitidis	*ctrA*	GCTGCGGTAGGTGGTT CAA	TTGTCGCGGATTTGCAA CTA	FAM-CATTGCCACGTGTCAGCTGC ACAT-TAMRA	(Corless et al. 2001)
Streptococcus suis serotype 2	*cps2J*	GGTTACTTGCTACTTTT GATGGAAATT	CGCACCTCTTTTATCTCT TCCAA	FAM-TCAAGAATCTGAGCTGCAAA AGTGTCAAATTGA -TAMRA	(Nga et al. *in press*)

Herpes simplex 1/2	*gB*	CCGTCAGCACCTTCATCG A	CGCTGGACCTCCGTGTAGTC	FAM-CCA CGA GAT CAA GGA CAG CGG CC-TAMRA	(Jerome et al. 2002)
Enteroviruses	*5'UTR*	CCCTGAATGCGGCTAAT	ATTGTCACCATAAGCAGCC	FAM-CGG AAC CGA CTA CTT TGG GT-TAMRA	(Beld et al. 2004)
Phocid Herpes virus (PhHV) (*)	*gB polymerase*	GGGCGAATCACAGATTGAATC	GCGGTTCCAAACGTACCAA	Cy5-TTTTTATGTGTCCGCCACCATCTGGATC-BHQ3	(van Doornum et al. 2003)
Equine arteritis virus (EAV) (*)	*NSP*	CATCTCTTGCTTTGCTCCTTAG	AGCCGCACCTTCACATTG	Cy5-CGCGCTCGCTGTCAGAACAACATTATTGCCCACAGCGCG-BHQ3	(Scheltinga et al. 2005)

(*) PhHV was used as DNA internal control and EAV was used as RNA internal control.

2.6 Serological methods

Viet Nam is a place where Japanese encephalitis virus (JEV) and Dengue virus (DENV) co-circulate. To confirm Japanese encephalitis or Dengue encephalitis, we used JEV/DENV IgM ELISA assay of Venture Technologies Sdn Bhd (Malaysia), which utilizes inactivated antigens from JEV and DENV1-DENV4 and can distinguish IgM elicited by JEV from that elicited by DENV (Cardosa et al. 2002). ELISA protocol was constructed according to manufacturer's instructions.

2.6.1 Components of ELISA kit

Stored at room temperature

ELISA plates – 5 plates/pack	3 packs
ELISA plate covers – recyclable	1 piece
Phosphate buffered saline (PBS) salts (×10)	3 packets
Tween20	1 bottle
Stopping solution	1 bottle

Stored at 4^0C

ELISA grade BSA – 1g/tube	3 tubes
0.2M sodium carbonate	1 bottle
0.2M sodium bicarbonate	1 bottle
Anti-human μ-chain A425	1 vial
Monoclonal antibody	1 bottle
Conjugate P260	1 vial
Substrate tablets – 5 mgOPD/tablet	15 tablets

Substrate buffer	1 bottle
30% hydrogen peroxide	1 vial

Stored at -20^{0}C

Positive control sera	1 vial
Negative control sera	1 vial
Freeze-dried Dengue antigen	15 bottles
Freeze-dried JEV	15 bottles
Freeze-dried control antigen	15 bottles

2.6.2 Preparation of reagents

Preparation of coating buffer (0.05M carbonate-bicarbonate buffer, pH 9.6)

Mix: 1.6 ml 0.2M sodium carbonate

3.4 ml 0.2M sodium bicarbonate

15 ml water

Preparation of working phosphate buffered saline (PBS)

Dissolve one packet of PBS salts into 10 litres of water.

Store at room temperature

Preparation of 10% BSA-PBS

Add 10 ml PBS to 1 g BSA

Store frozen at -20^{0}C

Preparation of blocking buffer, 0.5% BSA-PBS

1 ml 10% BSA-PBS

19 ml PBS

Store frozen at -20^{0}C

Preparation of washing buffer, PBS-0.05% Tween20

1 ml Tween20

2 litres PBS

Store at room temperature

Preparation of diluents, 0.1% BSA-PBS

1 ml 10% BSA-PBS

99 ml PBS

Store frozen at -20^{0}C

2.6.3 ELISA protocol

There are 13 steps (see below).

1. Coating ELISA plate

   Dilute 5 μl anti-human μ-chain A425 in 10 ml coating buffer.

   Dispense 100 μl per well, cover plate and incubate overnight at 4^{0}C.

2. Blocking coated ELISA plate

   Aspirate the coating solution.

   Dispense 200 μl blocking buffer per well, incubate 2 hours at room temperature.

3. Wash plate 3 times with PBS-0.05% Tween20, soak for 1 minute in washing buffer between each wash.

4. Preparation of specimens, positive and negative control sera

   - CSF samples:

     100 μl CSF

     400 μl 0.1% BSA-PBS

   - Positive and negative controls:

     20 μl positive or negative control sera

     2 ml 0.1% BSA-PBS

   - Dispense 100 μl per well in triplicate for each specimen, three sets of triplicate for positive control and at least five sets of triplicate for negative control.

   - Incubate 2 hours at room temperature.

5. Wash plate 5 times with PBS-0.05% Tween20, soak for 1 minute in washing buffer between each wash

6. Freeze-dried antigen

   Reconstitute each vial of DENV antigen, JEV antigen and control antigen with 5 ml PBS and vortex.

   Dispense 100 μl per well, cover plate and incubate overnight at 4^0C (each specimen has 1 well DENV antigen, 1 well JEV antigen and 1 well control antigen).

7. Wash as for step 5

8. Monoclonal antibody mixture

   2.5 ml monoclonal antibody

   7.5 ml 0.1% BSA-PBS

   Mix well.

   Dispense 100 µl per well, incubate 1 hour at room temperature.

9. Wash as for step 5.

10. Conjugate

    Dilute 5 µl conjugate P260 in 10 ml 0.1% BSA-PBS, mix well.

    Dispense 100 µl per well, incubate 30 minute at room temperature in the dark.

11. Wash as for step 5

12. Preparation of substrate solution

    Dissolve 1 substrate tablet in 10 ml substrate buffer.

    Add 4 µl of 30% hydrogen peroxide just before use, mix well.

    Dispense 100 µl per well, incubate 30 minutes at room temperature in the dark.

13. Stopping solution

    Add 50 µl of stopping solution per well to stop reaction.

    Read absorbance at 492 nm.

2.6.4 Interpretations of IgM capture ELISA readings

1. Subtract the optical density (OD) value of the well containing control antigen (C) from the OD value of the well containing JEV antigen (AgJ) or DENV antigen (AgD) to get (AgJ-C) and (AgD-C) values. Calculate the mean of (AgD-C) and (AgJ-C) values for the negative controls.
2. The positive cut-off values were 5 times of mean of (AgD-C) value for negative controls in the case of Dengue and 5 times of mean of (AgJ-C) value for negative controls in the case of JE.
3. The OD values of the positive controls would be higher for DENV than for JEV because JEV positive antibodies are not as abundant as DENV positive antibodies.
4. For each CSF specimen, calculate (AgJ-C) and (AgD-C) values of specimen.

The unit (U) of ELISA test of CSF specimen was calculate as the below formula.

$$\frac{\textbf{OD value of CSF specimen}}{\textbf{Positive cutoff value}} \times 10$$

The result of ELISA was interpreted as below:

- Positive if titre of antibody was greater than 12 U.
- Negative if titre of antibody was less than 8 U.
- Indeterminate if titre of antibody was in the range of 8 to 12 U.

For each CSF specimen, we compared the results of DENV and JEV ELISAs. The pathogen against which the higher titre was recorded was assigned as the cause of infection.

Chapter 3

Aetiologies of central nervous system infection in Viet Nam

3.1 Introduction

Despite advances in antibiotic treatment and resuscitation, central nervous system (CNS) infection is still a serious and life-threatening disease, especially in the developing countries. It may manifest itself in a variety of clinical syndromes, including meningitis, encephalitis and focal central nervous system infections. According to the report of World Health Organization (WHO), the frequency of meningitis was 700,000 cases in 2004 and 70% of patients lived in Africa and South-east Asia (WHO 2008). Japanese encephalitis virus, an important cause of encephalitis in Asia, causes 50,000 encephalitis cases and 15,000 deaths annually and leaves many survivors with severe neurological and neuropsychiatric sequelae (Solomon et al. 2000). Tuberculosis is an important health problem in developing countries with 9.4 million cases reported in 2009 (WHO 2010). Tuberculous meningitis, whose case fatality rate was 65.3% in HIV-infected patients and 24.8% in uninfected patients in Viet Nam, is the most serious manifestation of this disease (Thwaites et al. 2004).

To reduce morbidity and mortality of CNS infection, epidemiological studies are needed to establish a rational policy for prevention and treatment. The initial choice of empirical antibiotics for the treatment of acute bacterial meningitis is usually pathogen-oriented. However, the relative frequencies of implicated pathogens of bacterial meningitis have varied over time, geographic regions and with age, underlying medical and/or surgical conditions of patients, and the routes by which the

pathogens are acquired (Lu et al. 2002). The most important pathogen of bacterial meningitis in children is *Haemophilus influenzae* type b, which caused 70% of bacterial meningitis among children under 5 year old in United States before the 1990s (Wenger et al. 1990; Dawson et al. 1999). The incidence of meningitis due to this organism in children aged 0 to 4 years before the conjugate vaccine era was 24 per 100,000 per year in United Kingdom (UK) and 54 per 100,000 per year in United States (US), but following an extensive vaccination programme the incidence fell to 0.6 per 100,000 per year and less than 1 per 100,000 per year, respectively (Peltola 2000). *Streptococcus pneumoniae* has become the most common cause of acute bacterial meningitis in developed countries. In adults, most epidemiological data on bacterial meningitis comes from the developed countries, in which the four most common infecting organisms are *Streptococcus pneumoniae* (30-60%), *Neisseria meningitidis* (13-37%), *Listeria monocytogenes,* and *Haemophilus influenzae* (Durand et al. 1993; Aronin et al. 1998; Boisson et al. 1999; Tang et al. 1999; Flores-Cordero et al. 2003; van de Beek et al. 2004). Nevertheless, *Neisseria meningitidis* is the main pathogen of bacterial meningitis in countries of sub-Saharan Africa, known as the Africa meningitis belt. It can cause epidemics, in which the disease incidence may approach 1,000/100,000 inhabitants (Campagne et al. 1999; van Deuren et al. 2000; Stephens et al. 2007). The outbreak of *Streptococcus suis* infection in human in July, 2005 in Sichuan, China with 215 cases and 38 deaths raised a question that whether this organism might be a neglected but important cause of CNS infection in Asia (Yu et al. 2006).

Viral encephalitis is one of the most challenging infectious diseases in which to establish the cause and hence design treatment and preventative strategies. The

only effective treatment is acyclovir in the early stage of *Herpes simplex* and varicella-zoster virus encephalitis. These pathogens caused 63/1570 (4%) of suspected encephalitis cases in the California Encephalitis Project, 48/203 (24%) of suspected encephalitis cases in UK and 75/253 (30%) of suspected encephalitis cases in France. No aetiology was found for 996/1570 (63%), 75/203 (37%) and 122/253 (48%) of cases, respectively (Glaser et al. 2006; Mailles et al. 2009; Granerod et al. 2010). Furthermore, aetiologies of encephalitis may be geographically different, such as Japanese encephalitis virus in Asia, West Nile virus in the United States and Murray Valley encephalitis virus in Australia (Solomon 2004).

The epidemiological data related to the aetiologies of central nervous system infections in Viet Nam is limited. The identification of the causes of CNS infections in Viet Nam is difficult for many reasons, including limited bacteriological culture facilities, lack of PCR and culture for viral identification and the use of antibiotics prior to presentation to hospital. In one report from the Hospital for Tropical diseases, *Streptococcus suis* caused 151/450 (33.6%) of adult bacterial meningitis, compared to 81/450 (18.0%) of *Streptococcus pneumoniae* (Mai et al. 2008). However from this data it is not possible to conclude that *Streptococcus suis* is an endemic disease because there was no epidemiological information from other provinces in Viet Nam, except Ha Noi (Wertheim et al. 2009). Until 2007, *Haemophilus influenzae* type b vaccine had not been introduced to the National Expanded Program on Immunization for children. The incidence of *H. influenzae* type b meningitis in children aged 0-5 years in Ha Noi, Viet Nam was 12 cases per 100,000 per year (Anh DD et al. 2006). Japanese encephalitis vaccine was introduced to the National Expanded Program on Immunization for children 1-5 years of age in the northern region in 1997. The

program was subsequently expanded to 437/676 districts of Viet Nam in 2007 (Yen NT et al. 2010). There is limited information on the incidence of Japanese encephalitis after introduction of vaccine, especially in the south of Viet Nam.

Understanding the aetiologies of central nervous system infection may help improve policy and practice for the treatment and prevention of these diseases. We conducted a prospective provincial hospital-based surveillance study to identify the causes of CNS infections across southern Viet Nam.

3.2 Aims

1. To describe the epidemiological characteristics of central nervous system infection in Viet Nam.
2. To identify the aetiologies of central nervous system infection in Viet Nam.
3. To calculate the incidence rates of central nervous system infection for each province.

3.3 Materials and Methods

Materials and methods are described in Section 2.3.1, 2.4.2, 2.4.4, 2.4.5, 2.5, and 2.6.

3.4 Results

Between August 2007 and April 2010, 1740 patients who fulfilled the inclusion criteria were admitted to 2 central, 10 provincial and 1 district hospitals.

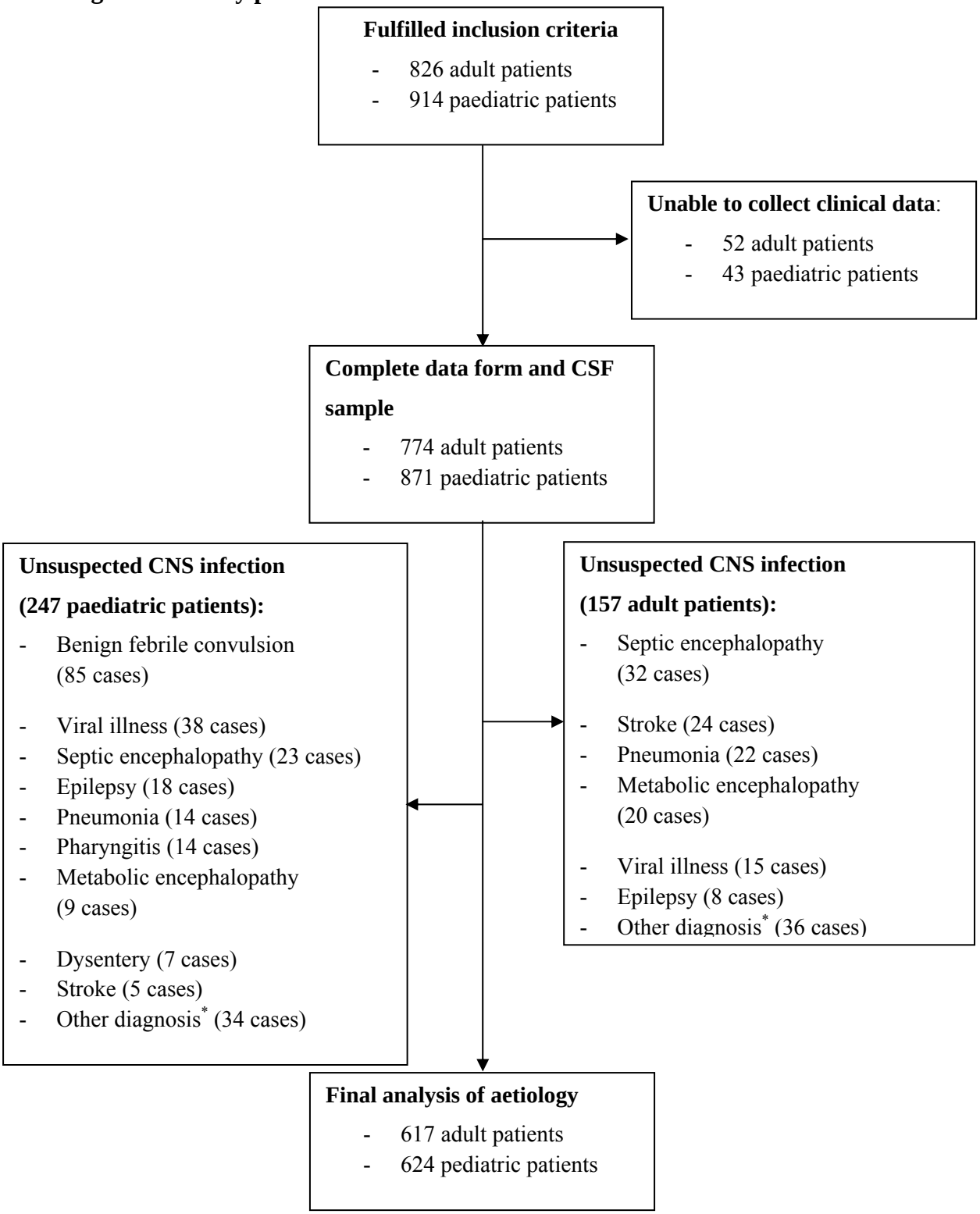
Clinical data were not available for 95 patients, including 52 adults and 43 children. In these 95 patients, pathogens were confirmed in 12/52 adults (eight *S. suis* serotype 2 cases, one *S. pneumoniae* case, one Japanese encephalitis case, one enteroviruses encephalitis case and one co-infection case of *S. suis* and enteroviruses) and in 13/43 children (four enteroviruses encephalitis cases, two Japanese encephalitis cases, two dengue encephalitis cases, two *H. influenza* type b meningitis cases, one meningococcal meningitis and two co-infection cases of *S. pneumoniae* and Japanese encephalitis virus).

After reviewing the clinical presentation and hospital tests of 1645 patients that had complete clinical data and CSF samples, I excluded 247 paediatric and 157 adult patients in the final analysis of aetiology (Figure 3-1). All of these patients had normal CSF parameters, no pathogen in CSF and blood and discharged diagnosis unrelated to CNS infection, such as benign febrile convulsion (in 85 children), epilepsy (24 cases), stroke (29 cases), metabolic encephalopathy (29 cases), septic encephalopathy (55 cases) and viral illness (53 cases). Finally, 1241 patients, including 617 adults and 624 children, were included in the aetiological analysis of CNS infection. The surveillance study was conducted over one year at six hospitals (Ca Mau, Bac Lieu, Tra Vinh, Can Tho, Sa Dec and Binh Phuoc) and over two years at the other seven hospitals (Soc Trang, Dong Thap, An Giang, Kien Giang, Dak Lak, Khanh Hoa and Hue). The number of patients recruited and the number of suspected CNS infection cases at each hospital are presented in Table 3-1. Four adult patients

had TBM score >6 and died but two cases, that had cloudy CSF (in which WC > 1000, high protein and low CSF glucose), were assigned as probable BM and two cases, that had clear CSF (in which normal CSF protein, CSF glucose and CSF lactate), were diagnosed as probable viral encephalitis. All other patients were classified according to Figure 3-2.

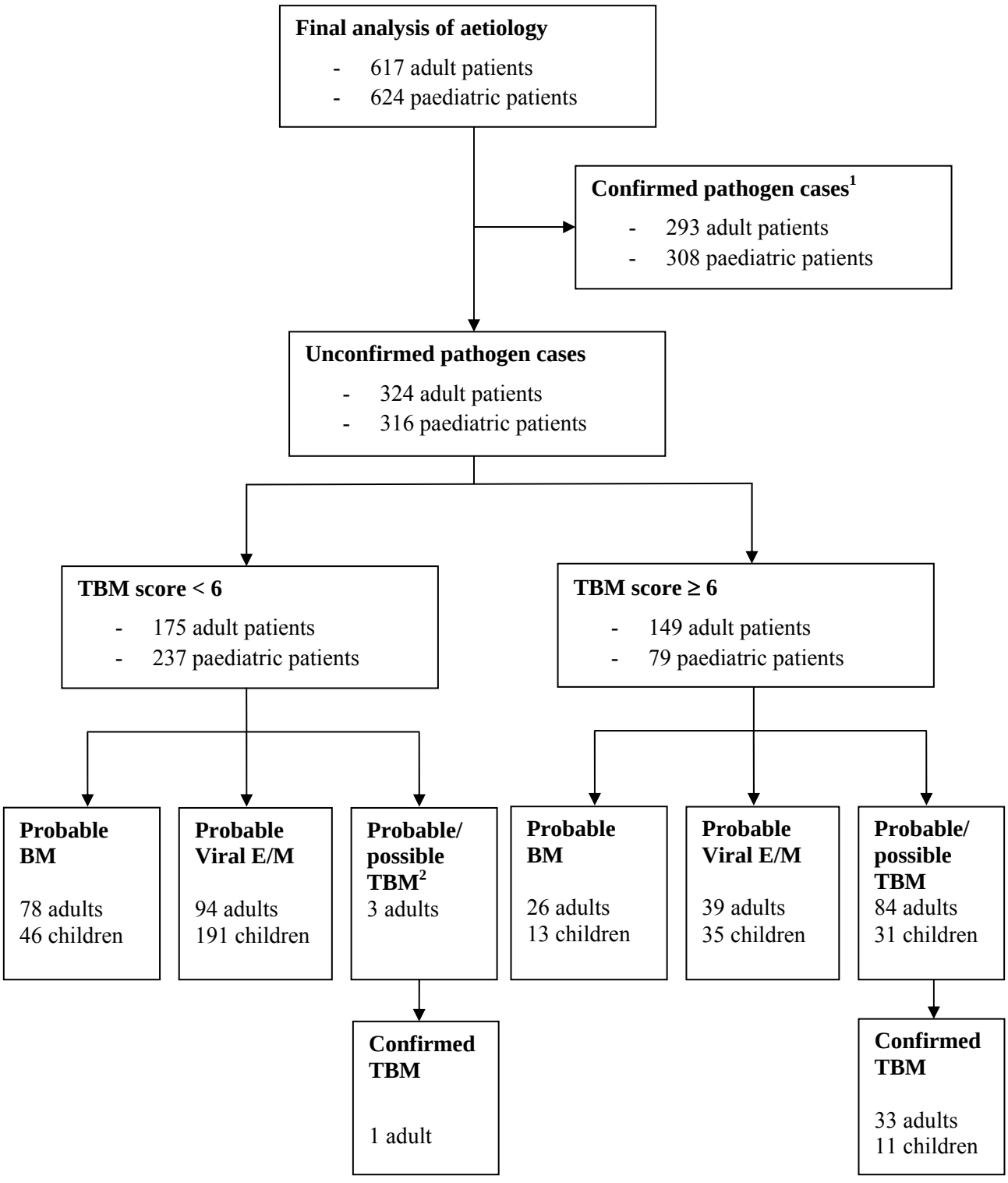
In 617 adults and 624 children suspected CNS infection, WC in CSF was missing in 19 adults and 14 children and WC was less than 10/μl in 54 adults and 115 children. Ten of these 73 adults and 2 of these 129 children had turbid CSF, in which 7 adults and 2 children were confirmed bacterial pathogens in CSF samples. Lactate in CSF sample was not measured in 439/617 adult patients and 483/624 paediatric patients.

Figure 3-1 Study profile



(*****) Other diagnosis included sinusitis (5 cases), brain tumours (9 cases), cerebral malaria (8 cases), mental disorders (7 cases), headache (7 cases), typhoid fever (5 cases), fever unknown origin (3 cases), autoimmune diseases (12 cases), diarrhoea (6 cases), lumbar disc herniation (2 cases), congenital heart diseases (2 cases), hydrocephalus (1 case), tetanus (1 case), severe anaemia (1 case) and chronic colitis (1 case).

Figure 3-2 Classification of central nervous system infection cases



[1] including 4 confirmed TBM cases co-infected with other pathogens

[2] Patients had typical manifestations of TBM, but short illness history.

Table 3-1Time frame and patients recruited at each hospital

Hospital	Participants	Time frame	Duration (months)	Patients recruited	Suspected CNS infection
Ca mau	Adults	Sep 2007 – Mar 2009	19	26	14
	Children	Feb 2008 – Mar 2009	14	26	25
Bac Lieu	Adults	Sep 2007 – Mar 2009	19	25	22
	Children	Feb 2008 – Aug 2008	7	17	14
Soc Trang	Adults	Sep 2007 – Apr 2010	32	100	83
	Children	Feb 2008 – Apr 2010	27	84	65
Can Tho	Adults	Sep 2009 – Aug 2010	12	52	48
	Children	-	-	-	-
Tra Vinh	Adults	Feb 2008 – Mar 2009	14	3	3
	Children	Feb 2008 – Mar 2009	14	24	22
Dong Thap	Adults	Jan 2008 – Mar 2010	27	37	29
	Children	Jan 2008 – Mar 2010	27	71	54
Sa Dec	Adults	Jan 2008 – Mar 2009	15	2	2
	Children	Jan 2008 – Mar 2009	15	56	32
An Giang	Adults	Mar 2008 – Mar 2010	25	67	59
	Children	Mar 2008 – Mar 2010	25	59	49
Kien Giang	Adults	Feb 2008 – Mar 2010	26	125	97
	Children	Mar 2008 – Mar 2010	25	66	47
Binh Phuoc	Adults	Mar 2008 – Mar 2009	13	16	10
	Children	Mar 2008 – Mar 2009	13	7	6
Dak Lak	Adults	Jan 2008 – Apr 2010	28	44	38
	Children	Jan 2008 – Apr 2010	28	137	104
Khanh Hoa	Adults	Nov 2007 – Apr 2010	30	51	30
	Children	Nov 2007 – Apr 2010	30	111	71
Hue	Adults	Apr 2008 – Apr 2010	25	226	182
	Children	Apr 2008 – Apr 2010	25	213	135

3.4.1 Characteristics of central nervous system infection patients

3.4.1.1 Baseline characteristics of CNS infection patients

Over 80% of CNS infection patients lived in rural areas and belonged to the Kinh ethnic group. The proportion of ethnic minorities in children group was higher than that in adult group (23.40% versus 14.42%, $p<0.001$). The overall ratio of male to female sex was 1.92/1.00. This ratio was highest in adult bacterial meningitis (3.03/1.00) and lowest in paediatric tuberculous meningitis (0.82/1.00). The aetiology of CNS infection in the children population was different to that in adult population. Viral encephalitis and meningitis made up nearly 70% of paediatric CNS infection, compared to 34% of adult CNS infection. Acute bacterial meningitis (BM) and tuberculous meningitis (TBM) presented with higher proportion in adult group (Table 3-2). In the children, BM patients were younger than TBM and viral encephalitis/meningitis, of which the median ages were 1.63, 3.00 and 5.00 years, respectively (Table 3-4). However, adult BM patients were older than adult TBM and viral encephalitis/meningitis (Table 3-3). The overall case fatality rate (CFR) at provincial hospital was 11.83% in adults compared to 6.73% in children. One third of TBM patients died before receiving anti-tuberculosis drugs (Table 3-2, 3-3 and 3-4).

Table 3-2 Characteristics of CNS infection patients

Characteristics	Adults (n=617)	Children (n=624)	p value[3]
Age (years), median (IQR)	38 (24; 52)	4 (1.29; 9)	_
Male, n (%)	431 (69.85)	385 (61.70)	0.002
Rural, n (%)	502 (81.36)	527 (84.86)	0.148
Regions, n (%)			<0.001[4]
- Mekong river delta (n=622)	353 (53.32)	309 (46.68)	-
- Central Viet Nam (n=415)	211 (50.84)	204 (49.17)	-
- Central highlands (n=142)	38 (26.76)	104 (73.24)	-
- South East (n=16)	10 (62.50)	6 (37.50)	-
- Others[1] (n=6)	5 (83.33)	1 (16.67)	-
Ethnicity, n (%)			
- Kinh	528 (85.58)	478 (76.60)	<0.001
- Khmer	64 (10.37)	68 (10.90)	0.764
- E de	10 (1.62)	27 (4.33)	0.005
- Raglai	3 (0.49)	14 (2.24)	0.008
- Others[2]	12 (1.94)	37 (5.93)	<0.001
HIV status, n (%)			
- Positive	10 (1.62)	0 (0)	-
- Negative	488 (79.09)	50 (8.01)	-
- Unknown	119 (19.29)	574 (91.99)	-

Diagnosis, n (%)			
- Bacterial meningitis	302 (48.95)	150 (24.04)	<0.001
- Viral encephalitis/ meningitis	209 (33.87)	432 (69.23)	<0.001
- Tuberculous meningitis	87 (14.10)	31 (4.97)	<0.001
- Eosinophilic meningitis	4 (0.65)	1 (0.16)	0.215
- Cryptococcal meningitis	2 (0.32)	-	0.247
- Cerebral toxoplasmosis	1 (0.16)	-	0.497
- Dual infection	12 (1.94)	10 (1.60)	0.648
Outcomes, n (%)			
- Alive	429 (69.53)	493 (79.01)	<0.001
- Died	73 (11.83)	42 (6.73)	0.002
- Transferred to other hospitals	88 (14.26)	71 (11.38)	0.128
- Unknown outcome	27 (4.38)	18 (2.88)	0.160

[1] Cambodia, Laos

[2] Cham, Co Tu, Dao, H'Mong, Jarai, M'Nong, Mong, Nung, Pa Ko, STieng, Ta Oi, Tay, Thai, Xo Dang, Van Kieu, Chinese and Laotian.

[3] Chi-squared test or Fisher's exact test (when one or more of the expected count is less than 5)

[4] Overall p-value for comparing age distribution (adults vs. children) between regions

Table 3-3 Characteristics of adult patients by diagnosis

Characteristics	Bacterial meningitis (n=302)	Viral encephalitis/ meningitis (n=209)	Tuberculous meningitis (n=87)
Age (years), median (IQR)[3]	45.5 (31;58)	28 (19; 43)	38 (25; 52)
Male, n (%)[4]	227 (75.17)	135 (64.59)	53 (60.92)
Rural, n (%)	246 (81.46)	166 (79.43)	74 (85.06)
Regions, n (%)			
- Mekong river delta (n=340)	158 (46.47)	118 (34.71)	64 (18.82)
- Central Viet Nam (n=209)	124 (59.33)	66 (31.58)	19 (9.09)
- Central highlands (n=35)	14 (40.00)	19 (54.29)	2 (5.71)
- South East (n=10)	5 (50.00)	4 (40.00)	1 (10.00)
- Others[1] (n=4)	1 (25.00)	2 (50.00)	1 (25.00)
Ethnicity, n (%)			
- Kinh	267 (88.41)	176 (84.21)	69 (79.31)
- Khmer	27 (8.94)	20 (9.57)	15 (17.24)
- E de	3 (0.99)	5 (2.39)	2 (2.30)
- Raglai	1 (0.33)	1 (0.48)	1 (1.15)
- Others[2]	4 (1.32)	7 (3.35)	-
Outcomes, n (%)			
- Alive	236 (78.15)	162 (77.51)	21 (24.14)
- Died[5]	30 (9.93)	16 (7.66)	24 (27.59)

- Transferred to other hospitals	24 (7.95)	21 (10.05)	37 (42.53)
- Unknown outcome	12 (3.97)	10 (4.78)	5 (5.75)

[1] Cambodia

[2] Co Tu, Dao, Chinese, Nung, Ta Oi, Thai, Van Kieu and STieng.

[3] Bacterial meningitis patients were older than viral encephalitis and TBM patients with $p<0.001$ and $p = 0.011$, respectively.

[4] Male sex proportion of BM patients was higher than the one of viral encephalitis and TBM patients with $p=0.010$ and $p=0.009$, respectively.

[5] Case fatality rate at provincial hospitals of TBM was higher than the one of BM and viral encephalitis/meningitis with OR=3.45; 95%CI [1.81; 6.58] and OR=4.60; 95%CI [2.18; 9.74]

Table 3-4 Characteristics of paediatric patients by diagnosis

Characteristics	Bacterial meningitis (n=150)	Viral encephalitis/ meningitis (n=432)	Tuberculous meningitis (n=31)
Age (years), median (IQR)[3]	1.63 (0.5; 6)	5 (2; 9)	3 (1.33; 9)
Male, n (%)	89 (59.33)	273 (63.19)	14 (45.16)
Rural, n (%)	124 (82.67)	366 (84.72)	27 (87.10)
Regions, n (%)			
- Mekong river delta (n=305)	55 (18.03)	235 (77.05)	15 (4.92)
- Central Viet Nam (n=200)	69 (34.50)	118 (59.00)	13 (6.50)
- Central highlands (n=101)	26 (25.74)	73 (72.28)	2 (1.98)
- South East (n=6)	0(0)	5 (83.33)	1 (16.67)
- Others[1] (n=1)	0(0)	1 (100.00)	0(0)
Ethnicity, n (%)			
- Kinh	125 (83.33)	326 (75.46)	21 (67.74)
- Khmer	8 (5.33)	55 (12.73)	4 (12.90)
- E de	8 (5.33)	17 (3.94)	-
- Raglai	3 (2.00)	7 (1.62)	4 (12.90)
- Others[2]	6 (4.00)	27 (6.25)	2 (6.45)
Outcomes, n (%)			
- Alive	127 (84.67)	352 (81.84)	4 (12.90)
- Died[4]	10 (6.67)	20 (4.63)	12 (38.71)

- Transferred to other hospitals	11 (7.33)	47 (10.88)	12 (38.71)
- Unknown outcome	2 (1.33)	13 (3.01)	3 (9.68)

[1] Cambodia

[2] Co Tu, Dao, H'Mong, Jarai, M'Nong, Mong, Nung, Pa Ko, STieng, Ta Oi, Tay and Thai.

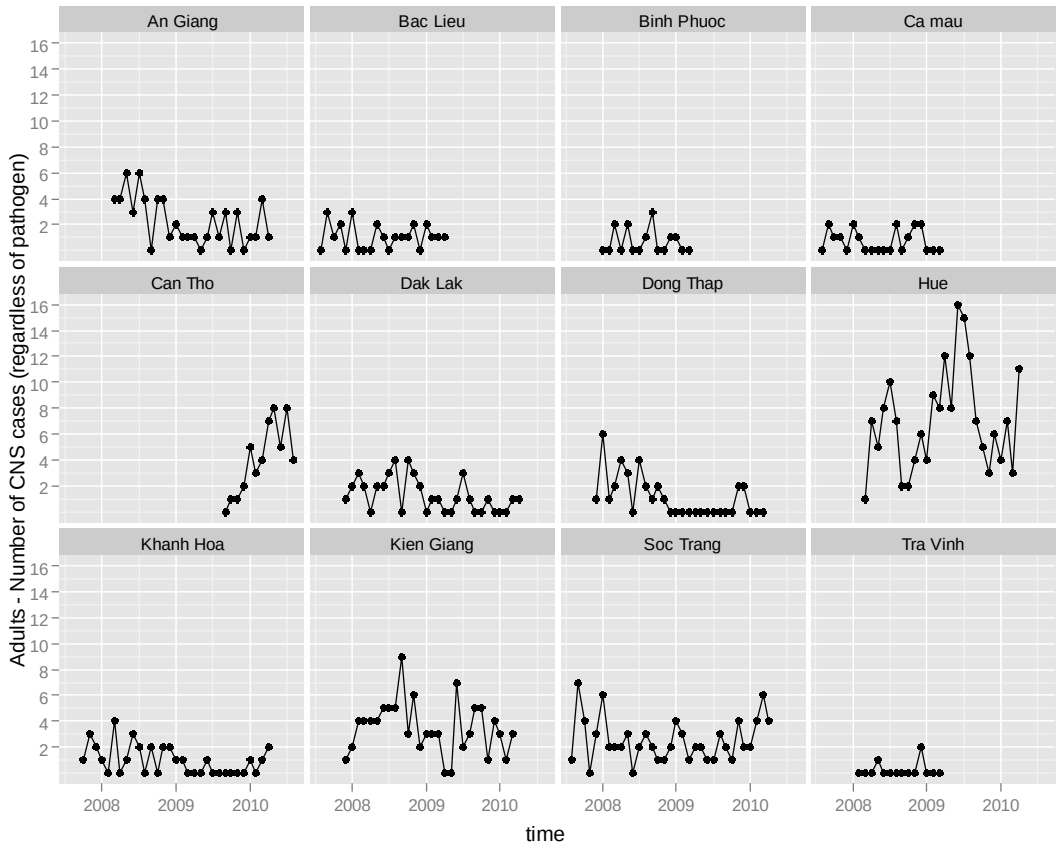
[3] Bacterial meningitis patients were younger than viral encephalitis and TBM patients with $p<0.001$ and $p = 0.046$, respectively

[4] Case fatality rate at provincial hospitals of TBM was higher than the one of BM and viral encephalitis/meningitis with OR=8.84; 95%CI [3.05; 26.01] and OR=13.01; 95%CI [5.12; 33.07].

3.4.1.2 Seasonality of CNS infection

Admission of adult patients with CNS infection had a seasonal pattern ($p<0.01$), with a peak month in June and an amplitude of difference (peak month vs. average) was + 21% (95%CI [+8%; 36%]). Linear time trend of infection decreased 13% per year (95%CI [-23%; -1%], $p<0.05$) but it was not significant if adjusted for over dispersion ($p=0.06$). However, the seasonal pattern was driven by Hue Central hospital alone. Test for seasonality was significant in Hue hospital ($p<0.001$) while it was not significant in all other hospitals ($p=0.36$) (Figure 3- 3).

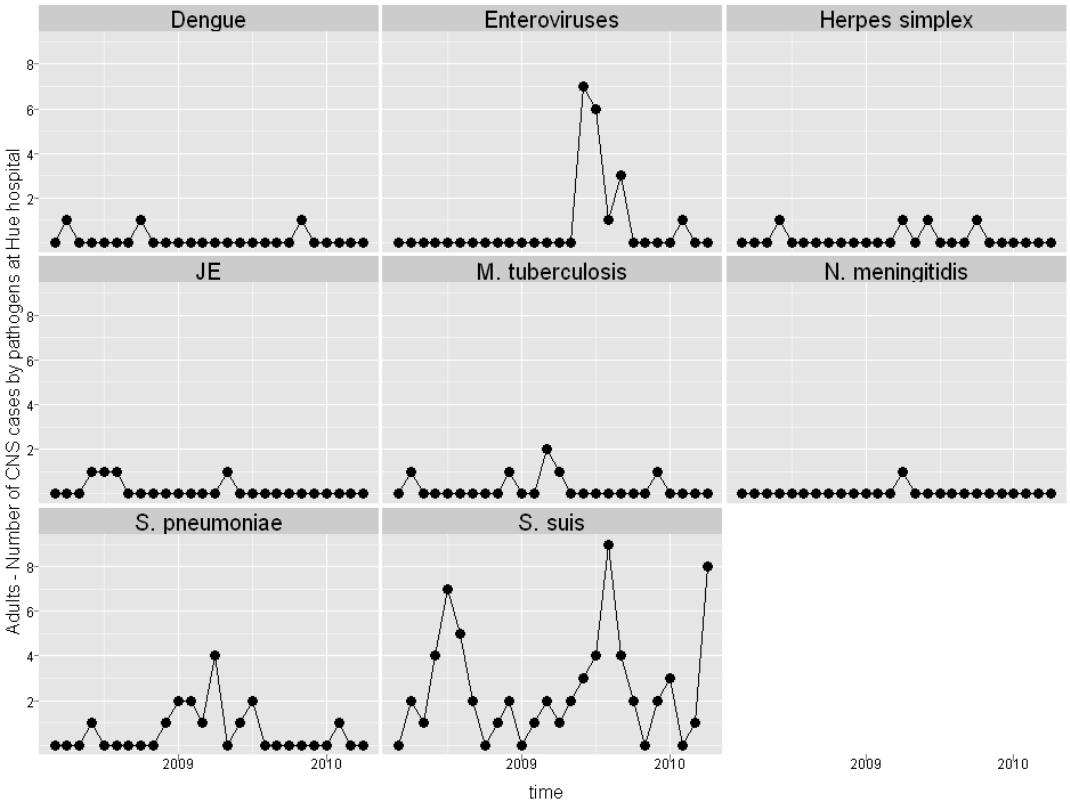
Figure 3-3 Time distribution of adult CNS infection admission by hospitals[1]



[1] "Dong Thap" includes Dong Thap provincial hospital and Sa Dec hospital

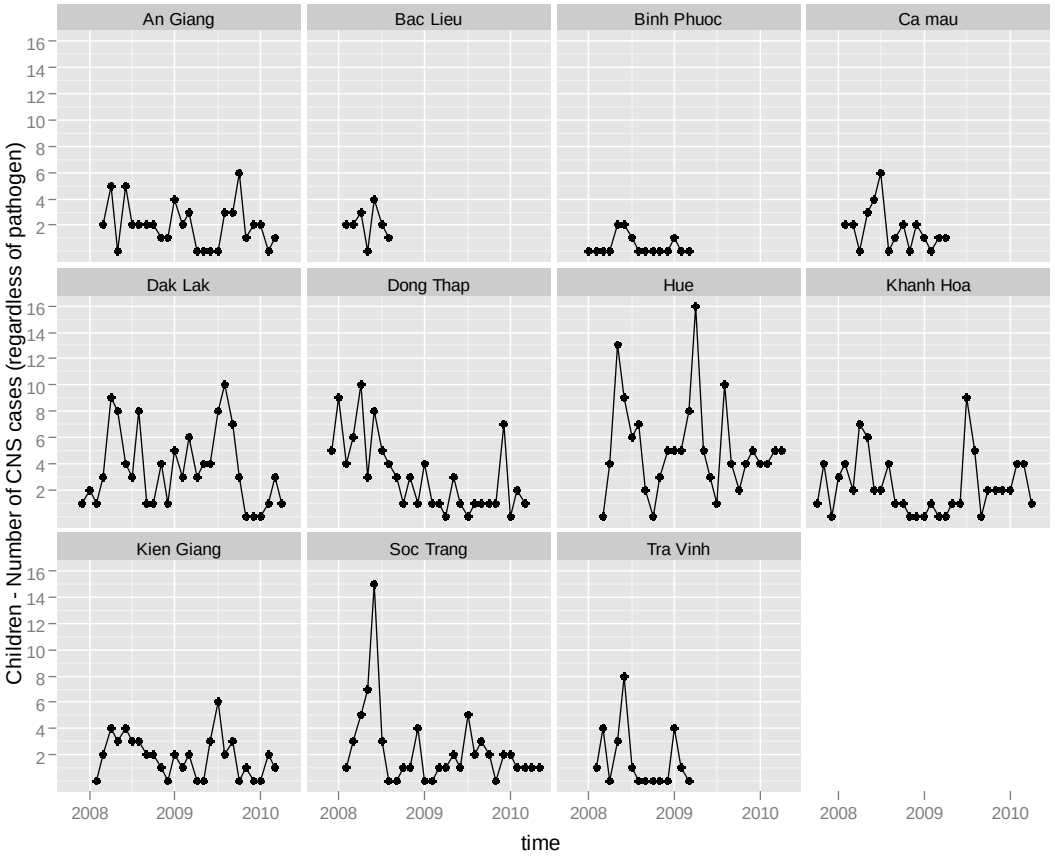
The seasonal change of CNS infection at Hue Central hospital seemed to be caused by infections caused by *Streptococcus suis* only (Figure 3-4).

Figure 3-4 Time distribution of adult CNS infection admission by all major pathogens at Hue Central hospital



The seasonal pattern of paediatric CNS infection admissions was also noted ($p<0.001$). The admission to hospitals peaked in June and had an amplitude of difference (peak month vs. average) of +51% (95%CI [+34%; +69%]) and linear time trend of -24% per year (95% CI [-33%; -14%], $p<0.001$) (Figure 3-5).

Figure 3-5 Time distribution of paediatric CNS infection admission by hospitals



3.4.1.3 Incidence rate of CNS infection

The incidence rate of CNS infection ranged from 1.86 cases per 100,000 person-years in Binh Phuoc province to 9.94 cases per 100,000 person-years in Thua Thien – Hue province. Age-specific incidence rates of CNS infection, of bacterial meningitis and of viral encephalitis/meningitis in Central Viet Nam were significantly higher than these rates in other regions, such as Mekong river delta, Central Highlands and South East. However, TBM's incidence rate was significantly lower in Central Highlands than in the Mekong river delta (adults) and Central Viet Nam (children) (Table 3-5, 3-6, 3-7, 3-8 and 3-9).

Table 3-5 Incidence rates of CNS infection by provinces

Province	Total person-time of observation ($\times 10^5$ person-years)	CNS infection		Confirmed (pathogen) cases		Unconfirmed (pathogen) cases	
		Number of patients	Incidence rate, 95%CI ($/10^5$ person-years)	Number of patients	Incidence rate, 95% CI ($/10^5$ person-years)	Number of patients	Incidence rate, 95%CI ($/10^5$ person-years)
Mekong river delta	**156.726**	**482**	**3.08 (2.81; 3.36)**	**250**	**1.60 (1.40; 1.81)**	**232**	**1.48 (1.30; 1.68)**
- Ca Mau	12.017	31	2.58 (1.75; 3.66)	22	1.83 (1.15; 2.77)	9	0.75 (0.34; 1.42)
- Bac Lieu[1]	6.470	9	1.39 (0.63; 2.64)	5	0.77 (0.25; 1.80)	4	0.62 (0.17; 1.58)
- Soc Trang	25.783	112	4.34 (3.58; 5.27)	55	2.13 (1.61; 2.78)	57	2.21 (1.67; 2.86)
- Can Tho[1]	9.303	21	2.26 (1.40; 3.45)	9	0.97 (0.44; 1.84)	12	1.29 (0.67; 2.25)
- Tra Vinh	10.008	24	2.40 (1.54; 3.57)	16	1.60 (0.91; 2.60)	8	0.80 (0.35; 1.58)
- Dong Thap	16.625	69	4.15 (3.23; 5.25)	34	2.05 (1.42; 2.86)	35	2.11 (1.47; 2.93)
- An Giang	42.918	91	2.12 (1.71; 2.60)	37	0.86 (0.61; 1.19)	54	1.26 (0.95; 1.64)
- Kien Giang	33.602	125	3.72 (3.10; 4.43)	72	2.14 (1.68; 2.70)	53	1.58 (1.18; 2.06)

Central Viet Nam	**44.826**	**291**	**6.49 (5.77; 7.28)**	**180**	**4.02 (3.45; 4.65)**	**111**	**2.48 (2.04; 2.98)**
- Khanh Hoa	23.090	75	3.25 (2.55; 4.07)	29	1.26 (0.84; 1.80)	46	1.99 (1.46; 2.66)
- Thua Thien-Hue	21.736	216	9.94 (8.66; 11.35)	151	6.95 (5.88; 8.15)	65	2.99 (2.31; 3.81)
Central highlands							
- Dak Lak	34.482	111	3.22 (2.65; 3.88)	49	1.42 (1.05; 1.88)	62	1.80 (1.38; 2.31)
South East							
- Binh Phuoc	8.580	16	1.86 (1.07; 3.03)	5	0.58 (0.19; 1.36)	11	1.28 (0.64; 2.29)
Overall	**244.614**	**900**	**3.68 (3.44; 3.93)**	**484**	**1.98 (1.81; 2.16)**	**416**	**1.70 (1.54; 1.87)**

[1] Only recruiting adult patients and calculating incidence rate per 100,000 person-years ≥ 15 years of age.

Table 3-6 Incidence rates of bacterial meningitis, viral encephalitis/meningitis and tuberculous meningitis by provinces[2]

Province	Total person-time of observation ($\times 10^5$ person-years)	Bacterial meningitis		Viral encephalitis / meningitis		Tuberculous meningitis	
		Number of patients	Incidence rate, 95%CI ($/10^5$ person-years)	Number of patients	Incidence rate, 95%CI ($/10^5$ person-years)	Number of patients	Incidence rate, 95%CI ($/10^5$ person-years)
Mekong river delta	**156.726**	**139**	**0.89 (0.75; 1.05)**	**268**	**1.71 (1.51; 1.93)**	**62**	**0.40 (0.30; 0.51)**
- Ca Mau	12.017	7	0.58 (0.23; 1.20)	22	1.83 (1.15; 2.77)	2	0.17 (0.02; 0.60)
- Bac Lieu [1]	6.470	2	0.31 (0.04; 1. 12)	1	0.15 (0.00; 0.86)	5	0.77 (0.25; 1.80)
- Soc Trang	25.783	27	1.05 (0.69; 1.52)	67	2.60 (2.01; 3.30)	18	0.70 (0.41; 1.10)
- Can Tho [1]	9.303	6	0.64 (0.24; 1.40)	10	1.07 (0.52; 1.98)	3	0.32 (0.07; 0.94)
- Tra Vinh	10.008	3	0.30 (0.06; 0.88)	17	1.70 (0.99; 2.72)	2	0.20 (0.02; 0.72)
- Dong Thap	16.625	23	1.38 (0.88; 2.08)	40	2.41 (1.72; 3.28)	5	0.30 (0.10; 0.70)
- An Giang	42.918	26	0.61 (0.40; 0.89)	53	1.23 (0.93; 1.62)	11	0.26 (0.13; 0.46)
- Kien Giang	33.602	45	1.34 (0.98; 1.79)	58	1.73 (1.31; 2.23)	16	0.48 (0.27; 0.77)

Central Viet Nam	**44.826**	**137**	**3.05 (2.57; 3.61)**	**128**	**2.86 (2.38; 3.40)**	**20**	**0.45 (0.27; 0.69)**
- Khanh Hoa	23.090	25	1.08 (0.70; 1.60)	41	1.78 (1.27; 2.41)	8	0.35 (0.15; 0.68)
- Thua Thien-Hue	21.736	112	5.15 (4.24; 6.20)	87	4.00 (3.21; 4.94)	12	0.55 (0.29; 0.96)
Central highlands							
- Dak Lak	34.482	31	0.90 (0.61; 1.28)	71	2.06 (1.61; 2.50)	3	0.09 (0.02; 0.25)
South East							
- Binh Phuoc	8.580	5	0.58 (0.19; 1.36)	9	1.05 (0.48; 1.99)	2	0.23 (0.03; 0.84)
Overall	**244.614**	**312**	**1.28 (1.14; 1.43)**	**476**	**1.95 (1.78; 2.13)**	**87**	**0.36 (0.28; 0.44)**

[1] Only recruiting adult patients and calculating incidence rate per 100,000 person-years ≥ 15 years of age.

[2] including both confirmed and probable cases and excluding co-infection cases.

Table 3-7 Incidence rates of CNS infection, bacterial meningitis, and viral encephalitis/meningitis in children by regions[9]

Province	Total person-time of observation (× 10^5 person-years)	CNS infection		Bacterial meningitis		Viral encephalitis/meningitis	
		Number of patients	Incidence rate, 95%CI (/10^5 person-years)	Number of patients	Incidence rate, 95% CI (/10^5 person-years)	Number of patients	Incidence rate, 95%CI (/10^5 person-years)
Mekong river delta	161.324 × 0.24	238	6.15 (5.39; 6.98)[1]	41	1.06 (0.76; 1.44)[4]	180	4.65 (3.99; 5.38)[6]
Central Viet Nam	44.826 × 0.27	138	11.40 (9.58; 13.47)	44	3.64 (2.64; 4.88)	81	6.69 (5.31; 8.32)
Central highlands	34.482 × 0.31	82	7.67 (6.10; 9.52)[2]	21	1.96 (1.22; 3.00)[5]	57	5.33 (4.04; 6.91)[7]
South East	8.580×0.29	6	2.41 (0.88; 5.25)[3]	-	-	5	2.01 (0.65; 4.69)[8]
Overall	249.212 × 0.26	464	7.16 (6.52; 7.84)	106	1.64 (1.34; 1.98)	323	4.98 (4.46; 5.56)

[1] In comparison with IR in Central Viet Nam, IRR (95%CI) = 0.54 (0.44; 0.66), p<0.001

[2] In comparison with IR in Central Viet Nam, IRR (95%CI) = 0.67 (0.51; 0.88), p=0.004

[3] In comparison with IR in Central Viet Nam, IRR (95%CI) = 0.24 (0.10; 0.53), p=0.001

[4] In comparison with IR in Central Viet Nam, IRR (95%CI) = 0.29 (0.19; 0.45), p<0.001

[5] In comparison with IR in Central Viet Nam, IRR (95%CI) = 0.54 (0.32; 0.91), p=0.020

[6] In comparison with IR in Central Viet Nam, IRR (95%CI) = 0.69 (0.53; 0.90), p=0.006

[7] In comparison with IR in Central Viet Nam, IRR (95%CI) = 0.80 (0.57; 1.12), p=0.189

[8] In comparison with IR in Central Viet Nam, IRR (95%CI) = 0.33 (0.14; 0.83), p=0.018

[9] including both confirmed and probable cases and excluding co-infection cases.

Table 3-8 Incidence rates of CNS infection, bacterial meningitis, and viral encephalitis/meningitis in adults by regions[10]

Province	Total person-time of observation (× 10^5 person-years)	CNS infection		Bacterial meningitis		Viral encephalitis/meningitis	
		Number of patients	Incidence rate, 95%CI (/10^5 person-years)	Number of patients	Incidence rate, 95% CI (/10^5 person-years)	Number of patients	Incidence rate, 95%CI (/10^5 person-years)
Mekong river delta	161.324 × 0.76	244	1.99 (1.75; 2.26)[1]	98	0.80 (0.65; 0.97)[4]	88	0.72 (0.58; 0.88)[7]
Central Viet Nam	44.826 × 0.73	153	4.68 (3.96; 5.48)	93	2.84 (2.29; 3.48)	47	1.44 (1.06; 1.91)
Central highlands	34.482 × 0.69	29	1.22 (0.82; 1.75)[2]	10	0.42 (0.20; 0.77)[5]	14	0.59 (0.32; 0.99)[8]
South East	8.580 × 0.71	10	1.64 (0.79; 3.02)[3]	5	0.82 (0.27; 1.92)[6]	4	0.66 (0.18; 1.68)[9]
Overall	249.212 × 0.74	436	2.36 (2.15; 2.60)	206	1.12 (0.97; 1.28)	153	0.83 (0.70; 0.97)

[1] In comparison with IR in Central Viet Nam, IRR (95%CI) = 0.43 (0.35; 0.52), p<0.001

[2] In comparison with IR in Central Viet Nam, IRR (95%CI) = 0.26 (0.18; 0.39), p<0.001

[3] In comparison with IR in Central Viet Nam, IRR (95%CI) = 0.35 (0.19; 0.67), p=0.001

[4] In comparison with IR in Central Viet Nam, IRR (95%CI) = 0.28 (0.21; 0.37), p<0.001

[5] In comparison with IR in Central Viet Nam, IRR (95%CI) = 0.15 (0.08; 0.28), p<0.001

[6] In comparison with IR in Central Viet Nam, IRR (95%CI) = 0.29 (0.12; 0.71), p=0.007

[7] In comparison with IR in Central Viet Nam, IRR (95%CI) = 0.50 (0.35; 0.71), p<0.001

[8] In comparison with IR in Central Viet Nam, IRR (95%CI) = 0.41 (0.23; 0.74), p=0.003

[9] In comparison with IR in Central Viet Nam, IRR (95%CI) = 0.46 (0.16; 1.27), p=0.133

[10] including both confirmed and probable cases and excluding co-infection cases.

Table 3-9 Incidence rates of tuberculous meningitis by regions[7]

Province	Total person-time of observation ($\times 10^5$ person-years)	Tuberculous meningitis					
		Adult patients			Pediatric patients		
		Proportion of adult group	Number of patients	Incidence rate, 95%CI ($/10^5$ person-years)	Proportion of children group	Number of patients	Incidence rate, 95%CI ($/10^5$ person-years)
Mekong river delta	161.324	0.76	48	0.39 (0.29; 0.52)[4]	0.24	14	0.36 (0.20; 0.61)[1]
Central Viet Nam	44.826	0.73	11	0.34 (0.17; 0.60)[5]	0.27	9	0.74 (0.34; 1.41)[2]
Central highlands	34.482	0.69	2	0.08 (0.01; 0.30)	0.31	1	0.09 (0.00; 0.52)
South East	8.580	0.71	1	0.16 (0.00; 0.91)[6]	0.29	1	0.40 (0.01; 2.24)[3]
Overall	249.212	0.74	62	0.34 (0.26; 0.43)	0.26	25	0.39 (0.25; 0.57)

[1] In comparison with IR in Central Highlands Viet Nam, IRR (95%CI) = 3.87 (0.51; 29.39), p=0.191

[2] In comparison with IR in Central Highlands Viet Nam, IRR (95%CI) = 7.95 (1.01; 62.74), p=0.049

[3] In comparison with IR in Central Highlands Viet Nam, IRR (95%CI) = 4.79 (0.30; 76.61), p=0.268

[4] In comparison with IR in Central Highlands Viet Nam, IRR (95%CI) = 4.66 (1.13; 19.16), p=0.033

[5] In comparison with IR in Central Highlands Viet Nam, IRR (95%CI) = 4.00 (0.89; 18.04), p=0.071

[6] In comparison with IR in Central Highlands Viet Nam, IRR (95%CI) = 1.95 (0.18; 21.54), p=0.585

[7] including both confirmed and probable or possible cases and excluding co-infection cases.

3.4.2 Aetiology of central nervous system infection

The aetiologies of CNS infection were identified in half of patients (Table 3-10), in which the most common pathogen was *Streptococcus suis* serotype 2 in adult group (23.82%) and Japanese encephalitis virus in children group (22.76%). *Mycobacterium tuberculosis* was confirmed in 34/617 (5.51%) adult patients and 11/624 (1.76%) paediatric patients. Dual infection was demonstrated in 22 cases, including 12 adults and 10 children. This condition was mainly related to dengue infection accompanying a bacterial pathogen (9/22 cases) or Japanese encephalitis and a bacterial pathogen (7/22 cases).

Table 3-10 Pathogens of central nervous system infection

Pathogen, n (%)	Adults (n=617)	Children (n=624)
Bacteria		
Streptococcus suis serotype 2	147 (23.82)	-
Streptococcus pneumoniae	35 (5.67)	37 (5.93)
Haemophilus influenzae type b	-	39 (6.25)
Neisseria meningitidis	4 (0.65)	6 (0.96)
Streptococcus spp	2 (0.32)	-
Staphylococcus spp	1 (0.16)	3 (0.48)
Escherichia coli	2 (0.32)	2 (0.32)
Acinetobacter spp	1 (0.16)	1 (0.16)
Klebsiella pneumoniae	5 (0.81)	1 (0.16)
Enterococcus spp	1 (0.16)	-
Salmonella spp	-	2 (0.32)
Virus		
Japanese Encephalitis (JE) virus	11 (1.78)	142 (22.76)
Dengue virus	23 (3.73)	14 (2.24)
Enteroviruses	20 (3.24)	36 (5.77)
Herpes simplex	22 (3.57)	14 (2.24)
Tuberculosis		
Mycobacterium tuberculosis	34 (5.51)	11 (1.76)
Fungi		
Cryptococcus neoformans	2 (0.32)	-

Dual infection		
Dengue virus + *S. suis* serotype 2	2 (0.32)	-
Dengue virus + *S. pneumoniae*	1 (0.16)	-
Dengue virus + *N. meningitidis*	1 (0.16)	-
Dengue virus + *H. influenzae* type b	-	3 (0.48)
Dengue virus + *M. tuberculosis*	2 (0.32)	-
Dengue virus + Eosinophilic meningitis[1]	2 (0.32)	
JE virus + *S. pneumoniae*	1 (0.16)	-
JE virus + *N.meningitidis*	-	1 (0.16)
JE virus + *H. influenzae* type b	-	3 (0.48)
JE virus + *Salmonella spp*	-	1 (0.16)
JE virus + *Staphylococcus spp*	-	1 (0.16)
Enteroviruses + *H. influenzae* type b	-	1 (0.16)
Enteroviruses + *M. tuberculosis*	1 (0.16)	-
K. pneumoniae + *M. tuberculosis*	1 (0.16)	-
K. pneumoniae + *Herpes simplex*	1 (0.16)	-
Unknown pathogen	295 (47.81)	306 (49.04)

[1] Number of eosinophils in CSF sample was 352/880 (40%) in one case and 330/1320 (25%) in another case.

3.4.3 Aetiology of bacterial meningitis

3.4.3.1 Pathogens of bacterial meningitis

Bacterial pathogens were identified in over 60% of bacterial meningitis cases by culture or real-time PCR method. *Streptococcus suis* serotype 2 caused meningitis in 50% of the adult bacterial meningitis group, which was four times higher than meningitis cases caused by *Streptococcus pneumoniae* in this population. However, this pathogen was not reported in the paediatric patients. In children, *Haemophilus influenzae* type b and *Streptococcus pneumoniae* were the main pathogens causing bacterial meningitis, which were responsible for more than 50% of paediatric bacterial meningitis cases (Table 3-11).

Table 3-11 Pathogens of bacterial meningitis (excluding dual infection cases)

Pathogen, n (%)	Adults (n=302)	Children (n=150)
Streptococcus suis serotype 2	147 (48.68)	-
Streptococcus pneumoniae	35 (11.59)	37 (24.67)
Haemophilus influenzae type b	-	39 (26.00)
Neisseria meningitidis	4 (1.32)	6 (4.00)
Streptococcus spp	2 (0.66)	-
Staphylococcus spp	1 (0.33)	3 (2.00)
Escherichia coli	2 (0.66)	2 (1.33)
Acinetobacter spp	1 (0.33)	1 (0.67)
Klebsiella pneumoniae	5 (1.66)	1 (0.67)
Enterococcus spp	1 (0.33)	-
Salmonella spp	-	2 (1.33)
Unknown pathogen	104 (34.44)	59 (39.33)

3.4.3.2 Age distribution of bacterial meningitis causes

Aetiology of bacterial meningitis varied clearly in age groups. *H. influenzae* type b was the most common pathogen in the children less than 1 year of age. It caused 40% of bacterial meningitis cases in this age group. Risk of infection decreased every one year of incremental age. Over 90% of *H. influenzae* type b cases

were reported in the first five years of life. In adult, *S. suis* serotype 2 only caused less than 10% of bacterial meningitis in young adult patients (<30 years of age). However, the risk of infection increased with age and was responsible for 60 % of bacterial meningitis cases after 30 years old. Twenty five percent of bacterial meningitis cases in children and young adults were caused by *S. pneumoniae* while it was reported in less than 10% of bacterial meningitis aetiologies after 30 years of age. Risk of pneumococcal infection decreased with age increased in both children and adults (Table 3-12, Table 3-13 and Figure 3-6).

Figure 3-6 Age distribution of bacterial meningitis cases by cause

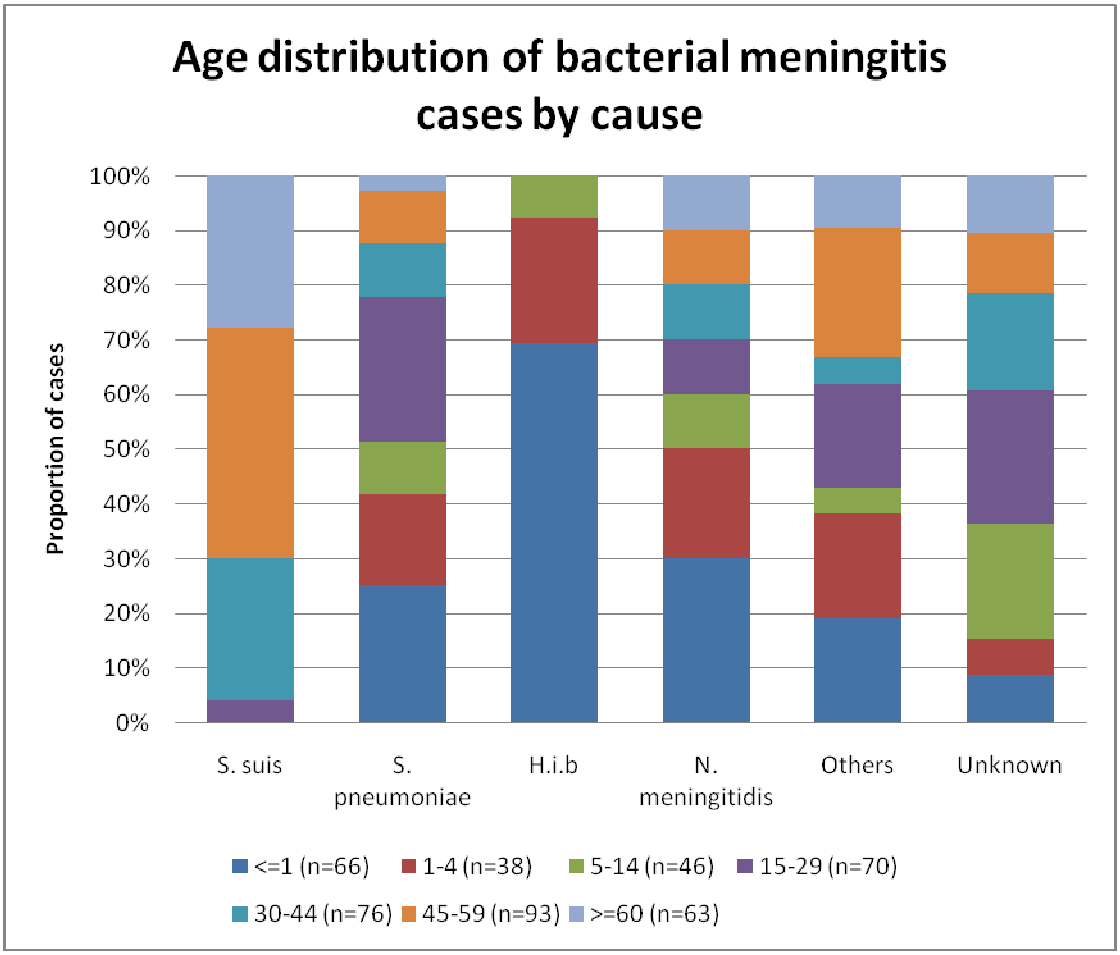


Table 3-12 Association between age and main pathogens of bacterial meningitis[1]

Pathogens	OR; 95% CI	p value
H. influenzae type b (in paediatric patients)		
- Age (by +1 year)	0.70; 95%CI [0.60; 0.83]	<0.001
Streptococcus suis serotype 2 (in adult patients)		
- Age (by +5 years)	1.30; 95%CI [1.23; 1.38]	<0.001
Streptococcus pneumoniae		
- Children: age (by +5 years)	0.46; 95%CI [0.27; 0.76]	0.003
- Adult: age (by +5 years)	0.89; 95%CI [0.80; 0.99]	0.028
N. meningitidis		
- Children: age (by +5 years)	0.56; 95%CI [0.18; 1.76]	0.319
- Adult: age (by +5 years)	1.10; 95%CI [0.86; 1.42]	0.452

[1] analyzed by logistic regression method.

Table 3-13 Pathogens of bacterial meningitis by age groups (excluding dual infection cases)

Pathogen, n (%)	Age group (years)						
	≤ 1 (n=66)	**1-4 (n=38)**	**5-14 (n=46)**	**15-29 (n=70)**	**30-44 (n=76)**	**45-59 (n=93)**	**≥ 60 (n=63)**
Streptococcus suis serotype 2	-	-	-	6 (8.57)	38 (50.00)	62 (66.67)	41 (65.08)
Streptococcus pneumoniae	18 (27.27)	12 (31.58)	7 (15.22)	19 (27.14)	7 (9.21)	7 (7.53)	2 (3.17)
Haemophilus influenzae type b	27 (40.91)	9 (23.68)	3 (6.52)	-	-	-	-
Neisseria meningitidis	3 (4.55)	2 (5.26)	1 (2.17)	1 (1.43)	1 (1.32)	1 (1.08)	1 (1.59)
Streptococcus spp	-	-	-	-	-	1 (1.08)	1 (1.59)
Staphylococcus spp	-	3 (7.89)	-	-	-	1 (1.08)	-
Escherichia coli	2 (3.03)	-	-	-	-	2 (2.15)	-
Acinetobacter spp	-	-	1 (2.17)	-	1 (1.32)	-	-

Klebsiella pneumoniae	-	1 (2.63)	-	3 (4.29)	-	1 (1.08)	1 (1.59)
Enterococcus spp	-	-	-	1 (1.43)	-	-	-
Salmonella spp	2 (3.03)	-	-	-	-	-	-
Unknown pathogen	14 (21.21)	11 (28.95)	34 (73.91)	40 (57.14)	29 (38.16)	18 (19.35)	17 (26.98)

3.4.3.3 Geographic distribution of bacterial meningitis causes

There was not any marked difference when analyzing geographic distribution of bacterial meningitis pathogens among Central Viet Nam, Mekong river delta and Central Highlands (Table 3-14 and Table 3-15).

Table 3-14 Aetiologies of adult bacterial meningitis by regions (excluding dual infection cases)[1]

Pathogen, n (%)	Adults			
	Mekong river delta (n=158)	**Central Viet Nam (n=124)**	**Central highlands (n=14)**	**South East (n=5)**
Streptococcus suis serotype 2 [2]	74 (46.84)	70 (56.45)	2 (14.29)	-
Streptococcus pneumoniae [3]	16 (10.13)	15 (12.10)	4 (28.57)	-
Haemophilus influenzae type b	-	-	-	-
Neisseria meningitidis [4]	3 (1.90)	1 (0.81)	-	-
Streptococcus spp	2 (1.27)	-	-	-
Staphylococcus spp	1 (0.63)	-	-	-
Escherichia coli	1 (0.63)	-	1 (7.14)	-
Acinetobacter spp	-	1 (0.81)	-	-
Klebsiella pneumoniae	1 (0.63)	4 (3.23)	-	-
Enterococcus spp	1 (0.63)	-	-	-
Salmonella spp	-	-	-	-
Unknown pathogen	59 (37.34)	33 (26.61)	7 (50.00)	5 (100.00)

[1] Comparison of proportions of each main pathogen (*S. suis, S. pneumoniae and N. meningitidis*) among regions

[2] p= 0.001

[3] p= 0.213

[4] p= 1.000

Table 3-15 Aetiologies of paediatric bacterial meningitis by regions (excluding dual infection cases)[1]

Pathogen, n (%)	Children			
	Mekong river delta (n=55)	Central Viet Nam (n=69)	Central highlands (n=26)	South East (n=0)
Streptococcus suis serotype 2	-	-	-	-
Streptococcus pneumoniae [2]	6 (10.91)	26 (37.68)	5 (19.23)	-
Haemophilus influenzae type b [3]	16 (29.09)	15 (21.74)	8 (30.77)	-
Neisseria meningitidis [4]	2 (3.64)	4 (5.80)	-	
Streptococcus spp	-	-	-	-
Staphylococcus spp	3 (5.45)	-	-	-
Escherichia coli	1 (1.82)	-	1 (3.85)	-
Acinetobacter spp	-	1 (1.45)	-	-
Klebsiella pneumoniae	-	1 (1.45)	-	-
Enterococcus spp	-	-	-	-
Salmonella spp	-	2 (2.90)	-	-
Unknown pathogen	27 (49.09)	20 (28.99)	12 (46.15)	-

[1] Comparison of proportions of each main pathogen (*S. suis, S. pneumoniae and N. meningitidis*) among regions

[2] p= 0.002

[3] p= 0.540

[4] p= 0.650

3.4.3.4 Incidence rate of common pathogens of bacterial meningitis

Incidence rate of *Streptococcus suis* serotype 2 in Thua Thien – Hue was significantly higher than other provinces. Rate ratio between Thua Thien – Hue and other provinces ranged from 3.6 to 25.5 times. The average incidence rates of this pathogen in adult population in Mekong river delta, Central Viet Nam and Central Highlands were 0.39, 1.68 and 0.13 per 100,000 person-years, respectively while these rates of *Streptococcus pneumoniae* meningitis were 0.10, 0.60 and 0.23 per 100,000 person-years. In children aged less than 5 years, average incidence rates of *H. influenzae* type b meningitis in Mekong river delta was lower than in Central Viet Nam and Central Highlands (1.06 vs. 3.35 and 1.61 per 100,000 children <5 years of age) (Table 3-16, 3-17 and 3-18).

Table 3-16 Incidence rates of *Streptococcus suis* meningitis by provinces

Province	Total person-time of observation ($\times 10^5$ person-years)	*Streptococcus suis*			
		Number of patients	Incidence rate, 95%CI ($/10^5$ person-years)	Proportion of population made up of adult[2]	Incidence rate in adult population, 95%CI ($/10^5$ person-years)
Mekong river delta	**161.324**	**48**	**0.30 (0.22; 0.39)**	**0.76**	**0.39 (0.29; 0.52)[3]**
- Ca Mau	12.017	3	0.25 (0.05; 0.73)	0.75	0.33 (0.07; 0.97)
- Bac Lieu[1]	6.470	1	0.15 (0.00; 0.86)	1	0.15 (0.00; 0.86)
- Soc Trang	25.783	10	0.39 (0.19; 0.71)	0.75	0.52 (0.25; 0.95)
- Can Tho[1]	9.303	4	0.43 (0.12; 1.10)	1	0.43 (0.12; 1.10)
- Tra Vinh	10.008	0	-	0.77	-
- Dong Thap	16.625	2	0.12 (0.01; 0.43)	0.76	0.16 (0.02; 0.57)
- An Giang	42.918	5	0.12 (0.04; 0.27)	0.76	0.15 (0.05; 0.36)
- Kien Giang	33.602	23	0.68 (0.43; 1.03)	0.74	0.92 (0.59; 1.39)

Central Viet Nam	**44.826**	**55**	**1.23 (0.92; 1.60)**	**0.73**	**1.68 (1.27; 2.19)**
- Khanh Hoa	23.090	3	0.13 (0.03; 0.38)	0.74	0.18 (0.04; 0.51)
- Thua Thien - Hue	21.736	52	2.39 (1.79; 3.14)	0.72	3.32 (2.48; 4.36)
Central highlands					
- Dak Lak	34.482	3	0.09 (0.02; 0.25)	0.69	0.13 (0.03; 0.37)[4]
South East					
- Binh Phuoc	8.580	0	-	0.71	-
Overall	**249.212**	**106**	**0.43 (0.35; 0.51)**	**0.74**	**0.57 (0.47; 0.70)**

[1] Only recruiting adult patients and calculating incidence rate per 100,000 person-years ≥ 15 years of age.

[2] Applying data of population by age group in the 2009 Vietnam population and house census (Table 2-2) (CP&HCSC 2010).

[3] In comparison with IR in Central Viet Nam, IRR (95%CI) = 0.24 (0.16; 0.35), p<0.001

[4] In comparison with IR in Central Viet Nam, IRR (95%CI) = 0.08 (0.02; 0.24), p<0.001

Table 3-17 Incidence rates of *Streptococcus pneumoniae* and *Neisseria meningitidis* meningitis by provinces

Province	Total person-time of observation ($\times 10^5$ person-years)	*Streptococcus pneumoniae*		*Neisseria meningitidis*	
		Number of patients	Incidence rate, 95%CI ($/10^5$ person-years)	Number of patients	Incidence rate, 95%CI ($/10^5$ person-years)
Mekong river delta	**156.726**	**15**	**0.10 (0.05; 0.16)[2]**	**5**	**0.03 (0.01; 0.07)[4]**
- Ca Mau	12.017	1	0.08 (0.00; 0.46)	0	-
- Bac Lieu[1]	6.470	0	-	0	-
- Soc Trang	25.783	1	0.04 (0.00; 0.22)	1	0.04 (0.00; 0.22)
- Can Tho[1]	9.303	1	0.11 (0.00; 0.60)	0	-
- Tra Vinh	10.008	0	-	0	-
- Dong Thap	16.625	5	0.30 (0.10; 0.70)	0	-
- An Giang	42.918	3	0.07 (0.01; 0.20)	0	-
- Kien Giang	33.602	4	0.12 (0.03; 0.30)	4	0.12 (0.03; 0.30)

Central Viet Nam	**44.826**	**27**	**0.60 (0.40; 0.88)**	**6**	**0.13 (0.05; 0.29)**
- Khanh Hoa	23.090	6	0.26 (0.10; 0.57)	2	0.09 (0.01; 0.31)
- Thua Thien - Hue	21.736	21	0.97 (0.60; 1.48)	4	0.18 (0.05; 0.47)
Central highlands					
- Dak Lak	34.482	8	0.23 (0.10; 0.46)[3]	0	-
South East					
- Binh Phuoc	8.580	0	-	0	-
Overall	**244.614**	**50**	**0.20 (0.15; 0.27)**	**11**	**0.04 (0.02; 0.08)**

[1] Only recruiting adult patients and calculating incidence rate per 100,000 person-years ≥ 15 years of age.

[2] In comparison with IR in Central Viet Nam, IRR (95%CI) = 0.16 (0.08; 0.30), p<0.001

[3] In comparison with IR in Central Viet Nam (IRR (95%CI) = 0.39 (0.17; 0.85), p=0.018

[4] In comparison with IR in Central Viet Nam (IRR (95%CI) = 0.24 (0.07; 0.78), p=0.018

Table 3-18 Incidence rates of *Haemophilus influenzae* type b meningitis by provinces

Province	Total person-time of observation (× 10^5 person-years)	*Haemophilus influenzae* type b				
		Number of patients		Incidence rate, 95%CI ($/10^5$ person-years)	Incidence rate[1] in children <15 year old, 95%CI ($/10^5$ person-years)	Incidence rate[1] in children <5 year old, 95%CI ($/10^5$ person-years)
		< 15 year old	<5 year old			
Mekong river delta	**140.953**	**12**	**12**	**0.09 (0.04; 0.15)**	**0.35 (0.18; 0.62)**	**1.06 (0.55; 1.86)[2]**
- Ca Mau	12.017	0	0	-	-	-
- Soc Trang	25.783	2	2	0.08 (0.00; 0.28)	0.31 (0.04; 1.12)	0.97 (0.12; 3.50)
- Tra Vinh	10.008	0	0	-	-	-
- Dong Thap	16.625	5	5	0.30 (0.10; 0.70)	1.25 (0.41; 2.92)	3.76 (1.22; 8.77)
- An Giang	42.918	2	2	0.05 (0.01; 0.17)	0.19 (0.02; 0.70)	0.58 (0.07; 2.10)
- Kien Giang	33.602	3	3	0.09 (0.02; 0.26)	0.34 (0.07; 1.00)	1.12 (0.23; 3.26)

Central Viet Nam	**44.826**	**12**	**12**	**0.27 (0.14; 0.47)**	**0.99 (0.51; 1.73)**	**3.35 (1.73; 5.85)**
- Khanh Hoa	23.090	1	1	0.04 (0.00; 0.24)	0.17 (0.00; 0.93)	0.54 (0.01; 3.02)
- Thua Thien - Hue	21.736	11	11	0.51 (0.25; 0.91)	1.81 (0.90; 3.23)	6.33 (3.16; 11.32)
Central highlands						
- Dak Lak	34.482	9	5	0.26 (0.12; 0.50)	0.84 (0.38; 1.60)	1.61 (0.52; 3.76)[3]
South East						
- Binh Phuoc	8.580	0	0	-	-	-
Overall	**228.841**	**33**	**29**	**0.14 (0.10; 0.20)**	**0.55 (0.38; 0.78)**	**1.58 (1.06; 2.27)**

[1] Applying data of population by age group in the 2009 Vietnam population and house census (Table 2-2) (CP&HCSC 2010)

[2] In comparison with IR in Central Viet Nam, IRR (95%CI) = 0.32 (0.14; 0.71), p=0.005

[3] In comparison with IR in Central Viet Nam, IRR (95%CI) = 0.47 (0.17; 1.33), p=0.155

3.4.4 Aetiology of viral encephalitis/meningitis

3.4.4.1 Pathogens of viral encephalitis/meningitis

Japanese encephalitis virus was the most common pathogen identified in patients with encephalitis and was responsible for 33% of paediatric viral encephalitis/meningitis cases. Dengue virus, enteroviruses and *Herpes simplex* virus were the common pathogens of adult viral encephalitis/meningitis, of which each pathogen caused 10% of infection. However, aetiology was not identified in 60% of viral encephalitis/meningitis population (Table 3-19).

Table 3-19 Pathogens of viral encephalitis/meningitis (excluding dual infection cases)

Pathogen, n (%)	**Adults (n=209)**	**Children (n=432)**	**p value**
Japanese encephalitis virus	11 (5.26)	142 (32.87)	<0.001
Dengue virus	23 (11.00)	14 (3.24)	<0.001
Enteroviruses	20 (9.57)	36 (8.33)	0.603
Herpes simplex	22 (10.53)	14 (3.24)	<0.001
Unknown pathogen	133 (63.64)	226 (52.31)	0.007

3.4.4.2 Age distribution of viral encephalitis/meningitis causes

Japanese encephalitis virus (JEV) was responsible for nearly 40% of encephalitis/meningitis cases in the age group 1-14, but it was less common in patients that were younger than 1 year of age or older than 15 years of age. Risk of JEV and enteroviruses infection increased with age in children but decreased after 15 years old. Though dengue encephalitis was more common in adults than children (11% versus 3.24%), trend of infection related to age was not statistically significant (Table 3-20, 3-21 and Figure 3-7).

Figure 3-7 Age distribution of viral encephalitis/meningitis cases by cause

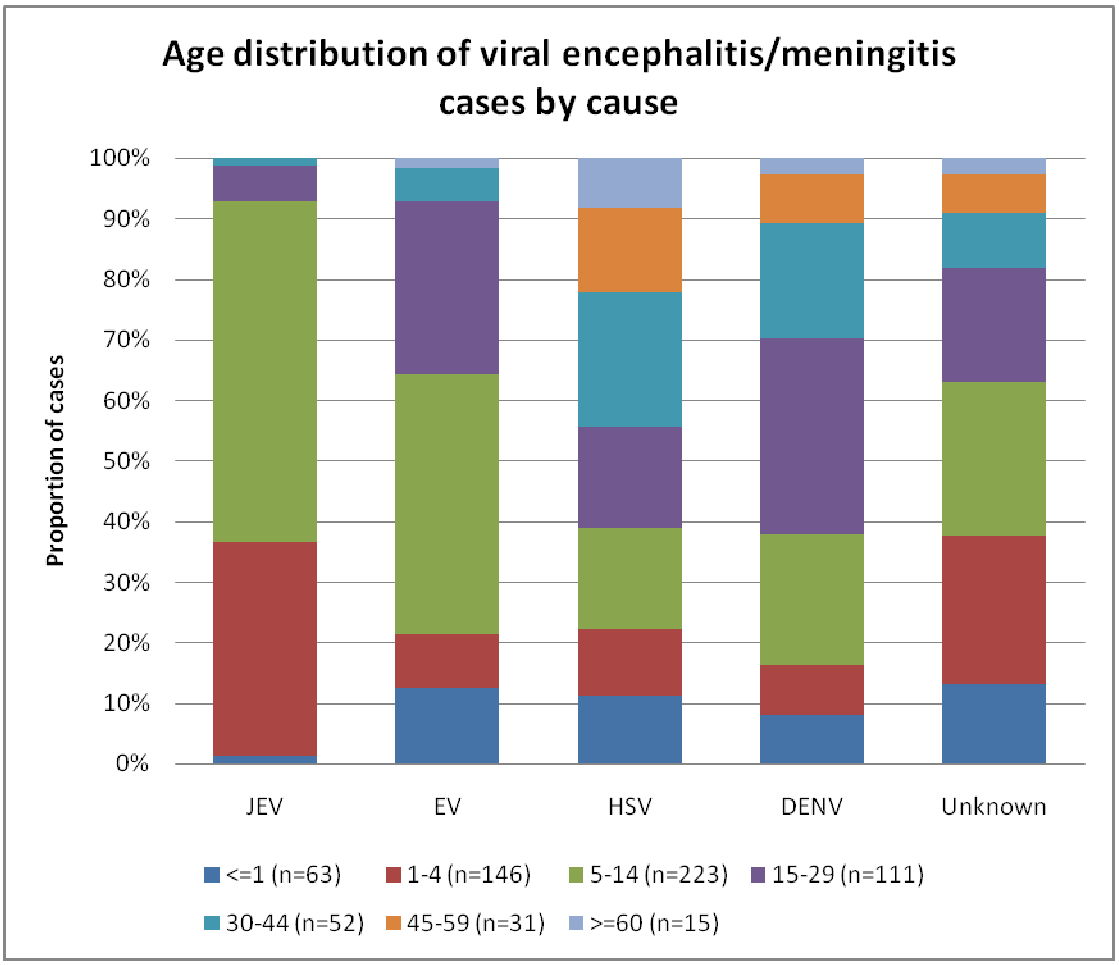


Table 3-20 Association between age and main pathogens of viral encephalitis/ meningitis[1]

Pathogens	OR; 95% CI	p value
Japanese encephalitis virus		
- Children: age (by + 5 years)	1.54; 95%CI [1.25; 1.89]	<0.001
- Adult: age (by +5 years)	0.63; 95%CI [0.45; 0.88]	0.007
Enteroviruses		
- Children: age (by +5 years)	1.49; 95%CI [1.04; 2.14]	0.030
- Adult: age (by +5 years)	0.65; 95%CI [0.52; 0.82]	<0.001
Herpes simplex		
- Children: age (by +5 years)	0.95; 95%CI [0.52; 1.75]	0.873
- Adult: age (by +5 years)	1.00; 95%CI [0.89; 1.13]	0.955
Dengue virus		
- Children: age (by +5 years)	0.95; 95%CI [0.52; 1.75]	0.869
- Adult: age (by +5 years)	0.89; 95%CI [0.78; 1.02]	0.087

[1] analyzed by logistic regression method.

Table 3-21 Aetiologies of viral encephalitis/meningitis by age groups (excluding dual infection cases)

Pathogen, n (%)	Age group (years)						
	≤ 1 (n=63)	1-5 (n=146)	6-14 (n=223)	15-29 (n=111)	30-44 (n=52)	45-59 (n=31)	≥ 60 (n=15)
Japanese encephalitis virus	2 (3.17)	54 (36.99)	86 (38.57)	9 (8.11)	2 (3.85)	-	-
Dengue virus	3 (4.76)	3 (2.05)	8 (3.59)	12 (10.81)	7 (13.46)	3 (9.68)	1 (6.67)
Enteroviruses	7 (11.11)	5 (3.42)	24 (10.76)	16 (14.41)	3 (5.77)	-	1 (6.67)
Herpes simplex	4 (6.35)	4 (2.74)	6 (2.69)	6 (5.41)	8 (15.38)	5 (16.13)	3 (20.00)
Unknown pathogen	47 (74.60)	80 (54.79)	99 (44.39)	68 (61.26)	32 (61.54)	23 (74.19)	10 (66.67)

3.4.4.3 Geographic distribution of viral encephalitis/meningitis causes

The aetiology of viral encephalitis/meningitis was rather homogenous among Mekong river delta, South East, Central Highlands and Central Viet Nam except enteroviruses caused 27% of viral aetiologies of adult encephalitis/meningitis in Central Viet Nam (Table 3-22 and Table 3-23).

Table 3-22 Aetiologies of adult viral encephalitis/meningitis by regions (excluding dual infection cases)

Pathogen, n (%)	Adults			
	Mekong river delta (n=118)	**Central Viet Nam (n=66)**	**Central highlands (n=19)**	**South East (n=4)**
Japanese encephalitis virus	4 (3.39)	4 (6.06)	3 (15.79)	-
Dengue virus	16 (13.56)	5 (7.58)	1 (5.26)	1 (25.00)
Enterovirus	2 (1.69)	18 (27.27)	-	-
Herpes simplex	18 (15.25)	4 (6.06)	-	-
Unknown pathogen	78 (66.10)	35 (53.03)	15 (78.95)	3 (75.00)

Table 3-23 Aetiologies of paediatric viral encephalitis/meningitis were stratified for regions (excluding dual infection cases)

Pathogen, n (%)	Children			
	Mekong river delta (n=235)	Central Viet Nam (n=118)	Central highlands (n=73)	South East (n=5)
Japanese Encephalitis virus	83 (35.32)	41 (34.75)	15 (20.55)	3 (60.00)
Dengue virus	8 (3.40)	4 (3.39)	1 (1.37)	1 (20.00)
Enterovirus	20 (8.51)	10 (8.47)	6 (8.22)	-
Herpes simplex	10 (4.26)	2 (1.69)	2 (2.74)	-
Unknown pathogen	114 (48.51)	61 (51.69)	49 (67.12)	1 (20.00)

3.4.4.4 Incidence rate of common pathogens of viral encephalitis/ meningitis

Incidence rate of Japanese encephalitis in children less than 15 years of age was approximately 4 per 100,000 person-years in Ca Mau, Soc Trang, Tra Vinh and Thua Thien - Hue province. Average incidence rate of this pathogen, which was 2.07, 2.56, 1.40 and 1.21 per 100,000 person-years in Mekong river delta, Central Viet Nam, Central Highlands and South East, was not significantly different among these surveillance regions. Incidence rates of other pathogens varied around 0.1 per 100,000 person-years in *Herpes simplex* and around 0.2 per 100,000 person-years in Dengue virus and enteroviruses, except incidence rate of enteroviruses in Thua Thien – Hue reached 0.97 per 100,000 person-years (Table 3-24, 3-25 and 3-26).

Table 3-24 Incidence rates of *Herpes simplex* and enteroviruses meningitis by provinces

Province	Total person-time of observation ($\times 10^5$ person-years)	*Herpes simplex*		Enteroviruses	
		Number of patients	Incidence rate, 95%CI ($/10^5$ person-years)	Number of patients	Incidence rate, 95%CI ($/10^5$ person-years)
Mekong river delta	**156.726**	**21**	**0.13 (0.08; 0.20)**	**18**	**0.11 (0.07; 0.18)**
- Ca Mau	12.017	1	0.08 (0.00; 0.46)	0	-
- Bac Lieu[1]	6.470	0	-	0	-
- Soc Trang	25.783	1	0.04 (0.00; 0.22)	5	0.19 (0.06; 0.45)
- Can Tho[1]	9.303	1	0.11 (0.00; 0.60)	0	-
- Tra Vinh	10.008	2	0.20 (0.02; 0.72)	1	0.10 (0.00; 0.56)
- Dong Thap	16.625	5	0.30 (0.10; 0.70)	4	0.24 (0.07; 0.62)
- An Giang	42.918	3	0.07 (0.01; 0.20)	4	0.09 (0.03; 0.24)
- Kien Giang	33.602	8	0.24 (0.10; 0.47)	4	0.12 (0.03; 0.30)

Central Viet Nam	**44.826**	**3**	**0.07 (0.01; 0.20)**	**22**	**0.49 (0.31; 0.74)**
- Khanh Hoa	23.090	0	-	1	0.04 (0.00; 0.24)
- Thua Thien - Hue	21.736	3	0.14 (0.03; 0.40)	21	0.97 (0.60; 1.48)
Central highlands					
- Dak Lak	34.482	2	0.06 (0.01; 0.21)	6	0.17 (0.06; 0.38)
South East					
- Binh Phuoc	8.580	0	-	0	-
Overall	**244.614**	**26**	**0.11 (0.07; 0.16)**	**46**	**0.19 (0.14; 0.25)**

[1] Only recruiting adult patients and calculating incidence rate per 100,000 person-years ≥ 15 years of age.

Table 3-25 Incidence rate of Japanese encephalitis by provinces

Province	Total person-time of observation ($\times 10^5$ person-years)	Japanese encephalitis				
		Number of patients	Incidence rate, 95%CI ($/10^5$ person-years)	Number of patients aged <15 years	Proportion of population made up of children aged <15 years[2]	Incidence rate in children <15 year old, 95%CI ($/10^5$ person-years)
Mekong river delta	**156.726**	**74**	**0.47 (0.37; 0.59)**	**70**	**0.24**	**2.07 (1.61; 2.61)[3]**
- Ca Mau	12.017	13	1.08 (0.58; 1.85)	12	0.25	3.99 (2.06; 6.98)
- Bac Lieu[1]	6.470	0	-	-	-	-
- Soc Trang	25.783	24	0.93 (0.60; 1.39)	24	0.25	3.72 (2.39; 5.54)
- Can Tho[1]	9.303	1	0.11 (0.00; 0.60)	-	-	-
- Tra Vinh	10.008	10	1.00 (0.48; 1.84)	10	0.23	4.34 (2.08; 7.99)
- Dong Thap	16.625	8	0.48 (0.21; 0.95)	8	0.24	2.00 (0.87; 3.95)
- An Giang	42.918	8	0.19 (0.08; 0.37)	7	0.24	0.68 (0.27; 1.40)
- Kien Giang	33.602	10	0.30 (0.14; 0.55)	9	0.26	1.03 (0.47; 1.96)

Central Viet Nam	**44.826**	**34**	**0.76 (0.53; 1.06)**	**31**	**0.27**	**2.56 (1.74; 3.64)**
- Khanh Hoa	23.090	9	0.39 (0.18; 0.74)	9	0.26	1.50 (0.69; 2.85)
- Thua Thien - Hue	21.736	25	1.15 (0.74; 1.70)	22	0.28	3.61 (2.27; 5.47)
Central highlands						
- Dak Lak	34.482	18	0.52 (0.31; 0.83)	15	0.31	1.40 (0.79; 2.31)[4]
South East						
- Binh Phuoc	8.580	3	0.35 (0.07; 1.02)	3	0.29	1.21 (0.25; 3.52)[5]
Overall	**244.614**	**129**	**0.53 (0.44; 0.63)**	**119**	**0.26**	**2.00 (1.66; 2.39)**

[1] Only recruiting adult patients and calculating incidence rate per 100,000 person-years ≥ 15 years of age.

[2] Data of population by age group in the 2009 Vietnam population and house census (Table 3-3) (CP&HCSC 2010)

[3] In comparison with IR in Central Viet Nam, IRR (95%CI) = 0.81 (0.53; 1.23), p=0.323

[4] In comparison with IR in Central Viet Nam, IRR (95%CI) = 0.55 (0.30; 1.01), p=0.056

[5] In comparison with IR in Central Viet Nam, IRR (95%CI) = 0.47 (0.14; 1.54), p=0.213

Table 3-26 Incidence rate of Dengue encephalitis by provinces

Province	Total person-time of observation ($\times 10^5$ person-years)	Dengue encephalitis	
		Number of patients	Incidence rate, 95%CI ($/10^5$ person-years)
Mekong river delta	**156.726**	**26**	**0.17 (0.11; 0.24)**
- Ca Mau	12.017	4	0.33 (0.09; 0.85)
- Bac Lieu[1]	6.470	1	0.15 (0.00; 0.86)
- Soc Trang	25.783	2	0.08 (0.01; 0.28)
- Can Tho[1]	9.303	1	0.11 (0.00; 0.60)
- Tra Vinh	10.008	0	-
- Dong Thap	16.625	2	0.12 (0.01; 0.43)
- An Giang	42.918	7	0.16 (0.07; 0.34)
- Kien Giang	33.602	9	0.27 (0.12; 0.51)
Central Viet Nam	**44.826**	**9**	**0.20 (0.09; 0.38)**
- Khanh Hoa	23.090	5	0.22 (0.07; 0.51)
- Thua Thien - Hue	21.736	4	0.18 (0.05; 0.47)
Central highlands			
- Dak Lak	34.482	4	0.12 (0.03; 0.30)
South East			
- Binh Phuoc	8.580	2	0.23 (0.03; 0.84)
Overall	**244.614**	**41**	**0.17 (0.12; 0.23)**

[1] Only recruiting adult patients and calculating incidence rate per 100,000 person-years ≥ 15 years of age.

3.4.5 Tuberculous meningitis

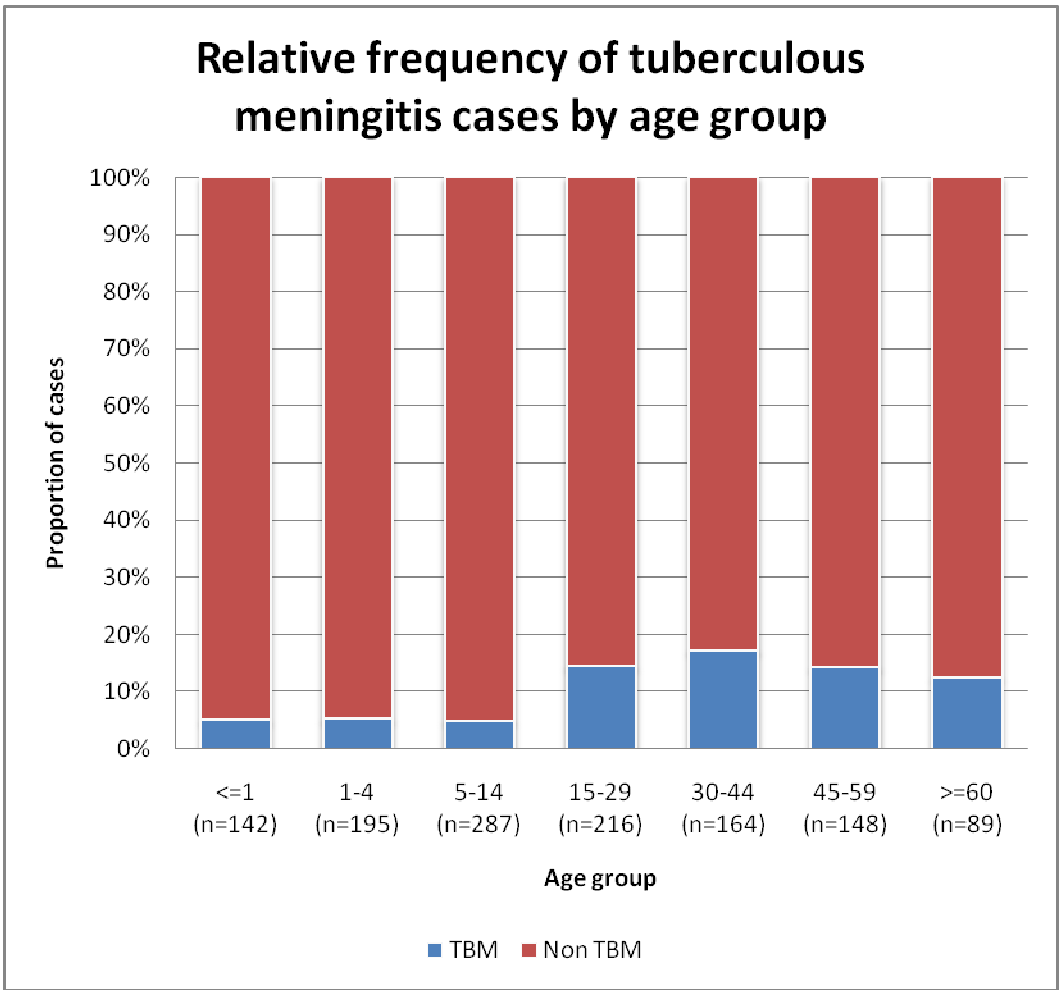
3.4.5.1 Classification of tuberculous meningitis

Tuberculous meningitis (TBM) was responsible for 10% (122/1241) of CNS infection cases (Table 3-2). Forty nine cases (40%) were confirmed by real-time PCR method. According to case definition of TBM, we classified 12/122 (10%) patients as probable TBM cases and 61/122 (50%) patients as possible TBM cases.

3.4.5.2 Age distribution of tuberculous meningitis cases

Proportion of TBM in paediatric CNS infection was 31/624 (4.97%) compared to 87/617 (14.10%) in adult patients ($p<0.001$) and these proportions were homogenous among age groups of children or adult population (Figure 3-8).

Figure 3-8 Relative frequency of tuberculous meningitis cases by age group



3.4.5.3 Geographic distribution of tuberculous meningitis cases

Two third (82/122) of TBM cases lived in Mekong river delta and adult TBM was more common in Mekong river delta than other regions (p=0.010) (Table 3-27).

Table 3-27 Epidemiological characteristics of TBM patients[1]

Region	Adults			Children		
	TBM	**Non-TBM**	**Total**	**TBM**	**Non-TBM**	**Total**
Mekong delta river	67	286	353	15	294	309
	(18.98)	(81.02)	(100.00)	(4.85)	(95.15)	(100.00)
Central Viet Nam	19	192	211	13	191	204
	(9.00)	(91.00)	(100.00)	(6.37)	(93.63)	(100.00)
Central Highlands	3	35	38	2	102	104
	(7.89)	(92.11)	(100.00)	(1.92)	(98.08)	(100.00)
South East	1	9	10	1	5	6
	(10.00)	(90.00)	(100.00)	(16.67)	(83.33)	(100.00)
Others[2]	1	4	5	0	1	1
	(20.00)	(80.00)	(100.00)	(0.00)	(100.00)	(100.00)

[1] Proportions of TBM in CNS infection were significantly different among regions of Viet Nam in adult population (p=0.010, Fisher's exact test), but were not significantly different in children population (p=0.178, Fisher's exact test).

[2] Laos and Cambodia

3.4.5.4 Incidence rate of tuberculous meningitis

Incidence rate of tuberculous meningitis in surveillance provinces ranged from 0.12 to 0.70 per 100,000 person-years but the average incidence rates of confirmed or unconfirmed TBM were not statistically different among Mekong river delta, Central Viet Nam, Central Highlands and South East (Table 3-6 and 3-28).

Table 3-28 Incidence rates of confirmed and probable/possible tuberculous meningitis (TBM) by provinces

Province	Total person-time of observation ($\times 10^5$ person-years)	Confirmed TBM		Probable/possible TBM	
		Number of patients	Incidence rate, 95%CI ($/10^5$ person-years)	Number of patients	Incidence rate, 95%CI ($/10^5$ person-years)
Mekong river delta	**156.726**	**31**	**0.20 (0.13; 0.28)**	**35**	**0.22 (0.16; 0.31)**
- Ca Mau	12.017	0	-	3	0.25 (0.05; 0.73)
- Bac Lieu[1]	6.470	4	0.62 (0.17; 1.58)	2	0.31 (0.04; 1.12)
- Soc Trang	25.783	8	0.31 (0.13; 0.61)	10	0.39 (0.19; 0.71)
- Can Tho[1]	9.303	1	0.11 (0.00; 0.60)	2	0.21 (0.03; 0.78)
- Tra Vinh	10.008	1	0.10 (0.00; 0.56)	1	0.10 (0.00; 0.56)
- Dong Thap	16.625	1	0.06 (0.00; 0.34)	4	0.24 (0.07; 0.62)
- An Giang	42.918	6	0.14 (0.05; 0.30)	5	0.12 (0.04; 0.27)
- Kien Giang	33.602	10	0.30 (0.14; 0.55)	8	0.24 (0.10; 0.47)

Central Viet Nam	**44.826**	**9**	**0.20 (0.09; 0.38)**	**11**	**0.25 (0.12; 0.44)**
- Khanh Hoa	23.090	3	0.13 (0.03; 0.38)	5	0.22 (0.07; 0.51)
- Thua Thien – Hue	21.736	6	0.28 (0.10; 0.60)	6	0.28 (0.10; 0.60)
Central highlands					
- Dak Lak	34.482	1	0.03 (0.00; 0.16)	3	0.09 (0.02; 0.25)
South East					
- Binh Phuoc	8.580	0	-	2	0.23 (0.03; 0.84)
Overall	**244.614**	**41**	**0.17 (0.12; 0.23)**	**51**	**0.21 (0.16; 0.27)**

[1] Only recruiting adult patients and calculating incidence rate per 100,000 person-years ≥ 15 years of age.

3.5 Discussion

This chapter describes the prospective provincial hospital-based surveillance study on central nervous system infection in the Central and the South Viet Nam, of which the average incidence was 3.71 per 100,000 person-years. *Streptococcus suis* serotype 2, which caused nearly half of adult bacterial meningitis, was the most common pathogen in adults while Japanese encephalitis virus was the most important cause of CNS infection in children. However, a viral pathogen although suspected was not confirmed in 60% of the viral encephalitis/meningitis patients.

3.5.1 Epidemiological characteristics of central nervous system infection

CNS infection was more common in the rural than in the urban areas because 83% of patients, in comparison with 75% of surveillance population, lived in the rural areas. Some pathogens, such as Japanese encephalitis virus, may need suitable environmental factors present in the rural areas for their transmission (Solomon 2004; CP&HCSC 2010). Infection susceptibility was higher in males than in females but the level of susceptibility varied in different types of CNS infection and age groups, in which male to female ratio ranged from 0.82 to 3.03 (Table 3-3 and 3-4). This ratio was similarly from 0.94 to 2.69 in other studies (Khwannimit et al. 2004; Thwaites et al. 2004; Glaser et al. 2006; Weisfelt et al. 2006; Nguyen et al. 2007; Afifi et al. 2009; Al-Tawfiq et al. 2009; Gurley et al. 2009; Mailles et al. 2009; Cho et al. 2010; Granerod et al. 2010). Reason of high proportion of male patients in CNS infection is unknown.

Viral encephalitis/meningitis was more common in children than in adults (69% vs. 34%). Children who had viral encephalitis/meningitis were also older than

children having bacterial meningitis. This phenomenon could be explained by different causes of CNS infection in different age groups (Peltola 2000; Solomon 2004; Yen NT et al. 2010). In the age group < 1 year, JE was documented in 2/142 patients, compared to 27/142 patients infected with *H. influeanzae* type b. However, the proportions were 86/287 and 3/287 in age group 5-15 years, respectively.

Seasonality of CNS infection was seen in both adult and paediatric group, in which June was the peak month. However, in the adult patients, it was only found at Hue Central hospital, where *Streptococcus suis* serotype 2 was responsible for nearly 40% of adult CNS infection. Japanese encephalitis was responsible for approximately 25% of paediatric CNS infection. These two pathogens, which tend to occur in the summer or the rainy season in the South-east Asia, could be the drivers of the seasonality of CNS infection (Kay et al. 1995; Solomon et al. 2000; Wangkaew et al. 2006; Wertheim et al. 2009). In China and Thailand, more *S. suis* patients were also admitted during rainy season, from June to September, than during the rest of the year. A parallel investigation by animal health authorities into illness and deaths of pigs occurring in the same region of China revealed evidence of *S. suis* infection (Kay et al. 1995; Huang et al. 2005; Wangkaew et al. 2006). There may be a high bacterial load in food items that are kept at high ambient temperatures or an increase in pig illnesses at the high ambient temperatures and humidity. Hence, *S. suis* increasingly transmits to humans in the hottest months when they directly contact with pigs or raw pork. In the case of JE, epidemiological studies have shown that after the monsoon rains Culex mosquitoes, the main vector of JE, breed prolifically and the transmission of virus is increase. In addition, temperature may be an important factor because the prolonged mosquito larval development time and longer extrinsic incubation period of

JEV at cooler temperature, which thus reduce the rate of virus transmission, could be another explanation (Solomon et al. 2000).

CNS infection is a serious group of diseases because of the high morbidity and mortality. Case fatality rate (CFR) in this study was 12% in adult group and 7% in children, but it widely varied among the pathogens causing the CNS infection. CFR ranged from 5-10% in bacterial meningitis and viral encephalitis/meningitis while it was 30-40% in tuberculous meningitis. This CFR was related to deaths before prescribing anti-tuberculous drugs because most Vietnamese tuberculous meningitis patients are admitted to an infectious diseases unit or a neurology unit in a general hospital before being transferred to a specialist tuberculosis unit or hospital. In addition, another 65% of HIV infected and 25% of HIV uninfected TBM patients died after receiving treatment (Thwaites et al. 2004). Therefore, TBM could be the most serious CNS infection in Viet Nam because of delayed diagnosis or misdiagnosis.

3.5.2 Aetiology of central nervous system infection

3.5.2.1 Bacterial meningitis

Streptococcus suis serotype 2 was responsible for causing 24% of all CNS infections and 49% of bacterial meningitis cases in adults. It has been the most important cause of adult acute bacterial meningitis in Viet Nam for the past fifteen years (Mai et al. 2008; Wertheim et al. 2009). The overall incidence rate of *Streptococcus suis* meningitis in the adult population (≥ 15 years of age) was 0.57 per 100,000 person-years but incidence rate of each surveillance province ranged from 0 to 3.32 per 100,000 person-years. The highest incidence rate was documented in Thua Thien – Hue province and *S. suis* meningitis was not reported in 2 provincial and 1 district hospitals, such as Tra Vinh, Binh Phuoc and Sa Dec (Table 3-16). However, lumbar puncture procedure was rarely performed in adult patients at Tra Vinh and Sa Dec hospital because the patient's relative often refused this procedure and requested that their relative be transferred to Hospital for Tropical diseases (HTD) in Ho Chi Minh city. In the same period of surveillance study (from August 2007 to April 2010), HTD received 5 *S. suis* meningitis cases from these hospitals (2 of Tra Vinh, 2 of Sa Dec and 1 of Binh Phuoc). Incidence rate of *S. suis* meningitis was twice that of *Streptococcus pneumoniae* meningitis (0.43 vs. 0.20 per 100,000 person-years), which is the most common cause of bacterial meningitis in European countries and United States (Durand et al. 1993; Gjini et al. 2006). It was also reported as a common cause of acute adult bacterial meningitis in other intensive pig rearing countries in Asia, such as Thailand and China, but it was a rare cause in European countries, the United States and Australia (Table 3-29) (Kay et al. 1995; Suankratay et al. 2004; Willenburg et al. 2006; Yu et al. 2006; Tramontana et al. 2008; van de Beek et al. 2008).

This surveillance study was conducted before *H. influenzae* type b conjugate vaccine was introduced to the National Expanded Program on Immunization in June 2010. This pathogen, which caused 39/150 (26%) of childhood bacterial meningitis cases, was the most common pathogen identified in children. This proportion was similar to results reported in another study from Ho Chi Minh City in 1990s and in other studies in the world before the conjugate vaccine era (Table 3-30). The highest incidence rate in children aged less than 5 years was reported in Thua Thien – Hue province (6.33 per 100,000 child-years), compared to 3.76 in Dong Thap province and 1.61 in Dak Lak province. The range of incidence rates among other surveillance studies in Asian countries was 0.98 to 28 per 100,000 child-years in children less than 5 years of age (Shetty et al. 2010). Our incidence rates of *H.* influenzae type b meningitis in children < 5 years of age were lower than the estimated incidence rate in Ha Noi City (12 per 100,000 child-years) and in Nha Trang City (18 per 100,000 child-years) (Anh DD et al. 2006; Anh et al. 2009). These two studies were conducted in the urban areas, where transmission rates of *H. influenzae* type b may be different to the rates in the rural areas.

Table 3-29 Pathogens of adult bacterial meningitis

Pathogens, n(%)	This study (n= 302)	Viet Nam[1] (n=450)	Singapore[2] (n=26)	Hongkong[3] (n=35)	Taiwan[4] (n=263)	Malawi[5] (n=427)	UK[6] (n=2977)	Neitherlands[7] (n=696)	US[8] (n=253)
S. suis	147 (49)	151 (34)	1 (4)	6 (17)	0 (0)	0 (0)	0 (0)	4 (1)	0 (0)
S. pneumoniae	35 (12)	81 (18)	4 (15)	7 (20)	64 (24)	275 (64)	1307 (44)	352 (51)	97 (38)
N. meningitidis	4 (1)	29 (7)	3 (12)	1 (3)	7 (3)	20 (5)	1177 (40)	257 (37)	35 (14)
H. influenzae	0 (0)	7 (2)	0 (0)	0 (0)	6 (2)	3 (1)	132 (4)	14 (2)	9 (4)
Listeria spp	0 (0)	0 (0)	2 (8)	2 (6)	6 (2)	0 (0)	127 (4)	30 (4)	29 (11)
Gram negative bacilli (*)	8 (3)	23 (5)	2 (8)	10 (28)	112 (43)	22 (5)	185 (6)	6 (1)	9 (4)
Other bacteria (**)	4 (1)	29 (6)	3 (11)	9 (26)	68 (26)	5 (1)	49 (2)	33 (5)	40 (16)
Unknown pathogens	104 (34)	130 (29)	11 (42)	N/A	N/A	102 (24)	N/A	N/A	34 (13)

(*) *E. coli, Klebsiella spp*, *Acinetobacter spp*

(**) Other *Streptococcus spp*, *Staphylococcus spp, Enterococcus spp*.

(1) (Mai et al. 2008)

(2) (Chan et al. 2002)

(3) (Hui et al. 2005)

(4) (Tang et al. 1999)

(5) (Scarborough et al. 2007)

(6) (Gjini et al. 2006)

(7) (van de Beek et al. 2004)

(8) (Durand et al. 1993)

Table 3-30 Pathogens of paediatric bacterial meningitis before introducing *H. influenzae* type b conjugate vaccine to EPI[#]

Pathogens, n(%)	This study (n= 150)	Viet Nam[1] (n=86)	Thailand[2] (n=67)	Saudi Arabia[3] (n=38)	Korea[4] (n=276)	Malawi[5] (n=267)	UK[5] (n=197)
H. influenzae type b	39 (26)	30 (35)	9 (13)	12 (32)	63 (23)	44 (16)	53 (27)
S. pneumoniae	37 (25)	24 (28)	1 (2)	13 (34)	91 (33)	62 (23)	22 (11)
N. meningitidis	6 (4)	-	3 (5)	7 (18)	16 (6)	7 (3)	111 (56)
Other bacteria (*)	9 (6)	11 (13)[$]	9 (13)	6 (16)	106 (38)	62 (24)	11 (6)
Unknown pathogens	59 (39)	21 (24)	45 (67)	N/A	N/A	92 (34)	N/A

(*) *E. coli, Klebsiella spp*, *Acinetobacter spp*, *Salmonella spp, Staphylococcus spp* and *Streptococcus spp*

(#) Expanded Program on Immunization

($) *N. meningitidis* and other bacteria.

(1) (Tran et al. 1998)

(2) (Muangchana et al. 2009)

(3) (Al-Tawfiq et al. 2009)

(4) (Cho et al. 2010)

(5) (Molyneux et al. 2006)

3.5.2.2 Viral encephalitis/meningitis

Diagnosis as well as identifying the causes of viral encephalitis/meningitis is one of the greatest challenges facing physicians. Many prospective surveillance studies have reported that at least 60% of patients hospitalized with encephalitis had no cause identified despite extensive laboratory testing (Glaser et al. 2006; Yong et al. 2008; Huppatz et al. 2009; Granerod et al. 2010). Our study, which was not an exception, focused on the common viral pathogens of encephalitis/ meningitis in Viet Nam, such as Japanese encephalitis, Dengue virus and enteroviruses, but the proportion with no pathogen identified was approximately 60% of all cases of encephalitis/meningitis (Table 3-19) (Ha et al. 1994; Solomon et al. 2000; Tu et al. 2007). However, this proportion might be reduced when immune-mediated causes, such as acute disseminated encephalomyelitis, N-methyl-D-aspartate (NMDA) receptor antibodies and voltage-gated potassium channel (VGKC) antibodies, were further investigated (Granerod et al. 2010). The aetiology of viral encephalitis /meningitis varies in different geographic settings, especially in the case of flaviviruses. Japanese encephalitis virus is the most important cause of encephalitis in South-east Asia. It was responsible for 24% of viral encephalitis/meningitis cases and over 90% of these cases were reported in children aged <15 years in this study. Japanese encephalitis rarely occurs in adults who live in endemic regions because serologic surveys have showed that almost everyone was exposed to virus during their childhood and is subsequently immune (Solomon 2004). Japanese encephalitis may be more common in the north of Viet Nam than in the central and south of Viet Nam. It caused 217/421 (50%) of acute encephalitis syndrome cases in five northern provinces (Yen NT et al. 2010). The range of incidence rates among the surveillance

provinces was 1.03 to 4.34 per 100,000 child-years in children less than 15 years of age but the average incidence rates of 4 regions, such as Mekong river delta, South East, Central Viet Nam and Central Highlands, were not statistically different (Table 3-25). Japanese encephalitis is the most common identified cause of encephalitis in Vietnamese children.

In 2009, over 100,000 dengue cases, which accounted for 40% of the Western Pacific Region's dengue cases, were reported in Viet Nam (WHO 2009). Dengue has become one of the most important epidemic diseases in this country. Neurological manifestations of dengue infection were reported in 0.5 – 6% of hospitalized dengue hemorrhagic fever patients (Hendarto et al. 1992; Solomon et al. 2000; Cam et al. 2001). Encephalopathy in dengue infection is well recognized but it remains unclear whether encephalopathy is caused by direct infection of virus in the central nervous system or indirectly via other mechanisms, such as liver failure, cerebral hypoperfusion, cerebral oedema and intracranial bleeding. However, detection of virus or anti-dengue IgM in the cerebrospinal fluid of a patient with encephalopathy after excluding other causes suggests that dengue virus may cause central nervous system infection (Varatharaj 2010). In this study, dengue virus, detected by the presence of specific IgM in CSF, was the only pathogen in 39/641 suspected viral encephalitis/meningitis cases (6.1%), including 25 adults and 14 children. Blood in the CSF sample (>5 red blood cells (RBC)/μl), which had the range of 12-38750 RBCs/μl, was found in 11/39 patients (data not shown). Our result are similar to other studies in the dengue endemic region, where the proportion of encephalitis cases related to dengue virus ranged from 4.2% to 47% (Solomon et al. 2000; Srey et al. 2002; Jackson et al. 2008; Le et al. 2010; Soares et al. 2011). Dengue should be

considered in patients who present with the clinical features of encephalitis, whether or not classical manifestations of dengue are present (Solomon et al. 2000; Varatharaj 2010).

Herpes simplex is one of the most common causes of encephalitis in European countries and in the United States, in which it caused 25% to 60% of confirmed viral encephalitis cases (Table 3-31). This pathogen was responsible for 6% of suspected viral encephalitis/meningitis cases in this study. Herpes simplex encephalitis was more common in adults than in children (10% vs. 3% of suspected viral encephalitis/ meningitis).

Enteroviruses were also amongst the most common causes of viral meningitis in Europe and North America (Lee et al. 2006; Ihekwaba et al. 2008). In this study, these viruses were reported as the second most common pathogen in both adults and children. However, 15/20 adult patients, compared to 6/36 paediatric patients, lived in Thua Thien – Hue province and 14/15 these adults, compared to none of the children, were admitted to Hue Central hospital in the period from June to September 2009 (Figure 3-4). This means most of adult enteroviruses encephalitis/meningitis cases observed in our study might be related to an outbreak in Thua Thien – Hue province in the summer of 2009.

Table 3-31 Viral pathogens of encephalitis/meningitis

Pathogens, n(%)	**This study (n= 641)**	**Viet Nam[1] (n=194)**(**)	**Cambodia[2] (n=81)**	**Malaysia[3] (n=220)**	**France[4] (n=90)**	**UK[5] (n=58)**	**US[6] (n=170)**
Japanese encephalitis virus (JEV)	153 (24)	50 (26)	16 (9)	- (***)	-	-	-
Dengue virus	37 (6)	9 (5)	5 (6)	-	-	-	-
Enteroviruses	56 (9)	18 (9)	0 (0)	9 (4)	2 (2)	3 (5)	43 (25)
Herpes simplex	36 (6)	1 (1)	0 (0)	21 (10)	55 (61)	38 (66)	45 (26)
Varicella-zoster virus	-	-	-	3 (1)	20 (22)	10 (17)	23 (14)
West Nile virus	-	-	-	-	1 (1)	-	19 (11)
Epstein-Barr virus	-	-	-	-	3 (3)	1 (2)	17 (10)
Cytomegalovirus	-	1 (1)	-	15 (7)	3 (3)	-	-
Other causes (*)	-	1 (1)	3 (4)	18 (8)	6 (7)	6 (10)	23 (14)
Unknown pathogen	359 (56)	114 (59)	57 (70)	154 (70)	N/A	N/A	N/A

(*) Measles, rabies, HIV, Hepatitis C virus, tick-borne encephalitis, Toscana virus, Influenza A, Human herpesvirus-6, rubella virus, parvovirus and JC virus.

(**) Only patients less than 16 years of age were recruited.

(***) RNA of JEV was detected in 10 patients' sera.

(1) (Le et al. 2010)

(2) (Srey et al. 2002)

(3) (Yong et al. 2008)

(4) (Mailles et al. 2009)

(5) (Granerod et al. 2010)

(6) (Glaser et al. 2006)

3.5.2.3 Tuberculous meningitis

Rapid and early diagnosis of tuberculous meningitis is fundamental to patient outcome. Physicians face numerous difficulties in establishing a TBM diagnosis, including the non-specific manifestations, low sensitivity of Ziehl-Neelsen staining and nucleic acid amplification and slowness of culture (Thwaites et al. 2000). To diagnose TBM in this study, we applied the consensus case definition of TBM, which included clinical, CSF, cerebral imaging information and could be used regardless of patient's age and HIV status (Marais et al. 2010). A commercial real-time PCR test, which had a sensitivity of 67% and the specificity of 94% after validation, was used to help confirm TBM. Ranking after *Streptococcus suis* serotype 2 meningitis and Japanese encephalitis, TBM, which was confirmed in 49/122 (40%) patients, was the third most common cause of CNS infection in Viet Nam. While TBM was the cause of 143/357 (40%) adult suspected CNS infection admitted to Hospital for Tropical Diseases (HTD), (Thwaites et al. 2002), the overall TBM proportion and its proportion stratified for adults and children were 10% (122/1241), 15% (91/617) and 5% (31/624), respectively in this study. TBM cases made up about 0.2% of total tuberculosis cases in the south of Viet Nam (Table 3-32). This is the first report of TBM in a context of CNS infection in Vietnamese provincial hospitals. The proportion of TBM at HTD was higher than those observed in our study because HTD receives TBM cases misdiagnosed as purulent bacterial meningitis from provincial hospitals. The protective role of BCG vaccination against TBM in children could explain why TBM was less common in children than in adults (Kumar et al. 2005; Trunz et al. 2006).

Table 3-32 Proportion of TBM in the total cases of tuberculosis in the south of Viet Nam

Province	TBM	Total TB cases[1]	Proportion of TBM (%, 95%CI)
Ca Mau	2	1326	0.15 (0.13-0.17)
Bac Lieu	5	998	0.50 (0.46-0.55)
Soc Trang	18	3664	0.49 (0.47-0.51)
Can Tho	3	2004	0.15 (0.13-0.17)
Dong Thap	5	2801	0.18 (0.16-0.19)
An Giang	11	9100	0.12 (0.11-0.13)
Kien Giang	16	5129	0.31 (0.30-0.33)
Tra Vinh	2	1405	0.14 (0.12-0.16)
Binh Phuoc	2	864	0.23 (0.20-0.27)
Total	**64**	**27291**	**0.23 (0.23-0.24)**

[1] Data from Report of Viet Nam National Tuberculosis Control Programme 2008 – 2009 (MoH 2008; MoH 2009)

3.5.2.4 Dual infections in CNS infection:

Dual infections in CNS infection, such as among members of herpesviruses or between a member of this group and a bacterium, were previously reported in both immunocompetent and immunocompromised patients by using PCR methods and/or intrathecal antibody determinations (Casas et al. 1996; Koskiniemi et al. 1996; Tang et al. 1997; Yamamoto et al. 2000; Weinberg et al. 2005; Vianello et al. 2008). In this study, we found 20 dual infection cases in 1241 suspected CNS infection patients. Most of these cases (18/20 cases) were infected with an endemic virus (Japanese encephalitis virus, dengue virus or enteroviruses) and a bacterium. In addition to 118 CSF samples of patients with suspected TBM according to the case definition, TBM PCR test was also done on another 10 CSF samples in which a pathogen had been identified, including 6 cases of dengue virus, 2 cases of *S. suis* serotype 2, 1 case of enteroviruses and 1 case of *K. pneumoniae.* Clinical manifestations and CSF parameters of these patients mimicked tuberculous meningitis. Four out of ten patients were found to contain *M. tuberculosis* DNA in their CSF samples (Table 3-10). Dual infection of JEV and enteroviruses or *H. influenzae* type b and enteroviruses was also reported in other studies in the South-east Asia (Ooi et al. 2007; Le et al. 2010). Our findings emphasize the role of these viruses as a co-infection pathogen in an endemic region.

3.5.2.5 Limitation of the study:

According to the report of the Ministry of Health on the HIV/AIDS status in Viet Nam, the total number of HIV infected and AIDS patients alive in the country in 2009 were 160,019 cases and 35,603 cases, respectively (MoH. 2010). Prevalence of HIV infection in Viet Nam was 187 cases per 100,000 inhabitants (MoH. 2010). *Cryptococcus neoformans* is one of the most important HIV-related opportunistic infections in the South-east Asia, where one third of CNS infection in HIV/AIDS patients related to this pathogen (Tansuphasawadikul et al. 1999; Ganiem et al. 2009; Park et al. 2009; Le et al. 2010). It can also be found in CNS infection in HIV uninfected patients in Viet Nam, in which 57 cases were admitted to Hospital for Tropical diseases (HTD) in a period of 10 years (Chau et al. 2010). However, only two cryptococcal meningitis cases were reported in our study. The unexpected rarity of this fungus could be explained by the limitation of microbiological culture facilities at the provincial hospitals or by the fact that lumbar punctures were not performed in HIV infected patients at these hospitals. There was only 10/617 (1.6%) HIV infected adult patients and none of paediatric patients with HIV infection recruited to study. In Viet Nam, attending physicians rarely do lumbar puncture on HIV patients at remote provincial hospitals because of their poor prognosis or relative's refusal. The number of TBM patients might be also underestimated because of low HIV prevalence of the study sample.

Another cause of CNS infection, eosinophilic meningitis, might also be under-reported. Fifty six adult patients with eosinophilic meningitis, who mostly lived in the Mekong river delta, were admitted to HTD between January 2002 and December 2005 (Le et al. 2007). The diagnosis relied on detection of eosinophil granulocytes in

CSF samples, but some provincial hospital's laboratories were unable to differentiate these cells from neutrophil granulocytes in CSF samples.

Confirmation of Japanese encephalitis and dengue encephalitis in our study relied on detection of specific IgM antibodies in CSF sample. Some CSF samples could have false negative result, especially in the early course of disease, because we only collected and tested the CSF samples on admission.

CNS infection is a serious disease in human, which has high mortality and morbidity. About 30% of patients admitted to hospital with JE die and half of the survivors have severe neurological sequelae, such as motor deficits, severe cognitive and language impairment (Solomon et al. 2000). In a randomized, double-blind, placebo-controlled trial of adjunctive dexamethasone in TBM treatment in Viet Nam, 199/545 (37%) and 56/545 (10%) of patients were dead or got severe disability nine months after treatment, respectively (Thwaites et al. 2004). Apart from the deafness of patients, lack of morbidity data (neurological sequelae) is another limitation of this study. Hearing loss was documented in 46/149 (31%) of *S. suis* patients, compared to 93/151 (62%) in a case-series study in Viet Nam (Mai et al. 2008). The higher rate of deafness in this case-series study could be explained by using audiogram testing.

Though CNS infection is serious enough to admit patients directly to provincial hospitals and most of CSF samples were taken there, this prospective hospital-based surveillance study could underestimate the incidence rates of CNS infection. Some patients that lived near a neighbouring provincial hospital could be admitted to this hospital, instead of to the provincial hospital of their province of residence. Some CSF samples may not have been collected because of deaths before admission, mild presentation of some viral meningitis cases, contraindications to

lumbar puncture procedure and the patients or relative's refusal for a lumbar puncture. The high use of antimicrobial agents in the community prior to admission could also decrease the possibility of bacterial isolation in the CSF samples. To overcome the limitations, we calculated incidence rates according to home address of patients and used real-time PCR method to identify the most common pathogens of bacterial meningitis in Viet Nam, such as *S. suis* serotype 2, *S. pneumoniae, H. influenzae* type b and *N. meningitidis*.

3.6 Conclusions

This is the first prospective provincial hospital-based surveillance study on the aetiology of central nervous system infection across Viet Nam. By using molecular diagnostic, serological and culture methods, we identified the pathogens in 640/1241 (52%) suspected CNS infection cases. The three most common pathogens in children were Japanese encephalitis virus, *Haemophilus influenzae* type b and *Streptococcus pneumoniae*. These organisms, which caused meningitis or encephalitis in at least one third of paediatric patients, could be prevented by implementing vaccination programmes with available vaccines in childhood. Meanwhile, *Streptococcus suis* serotype 2 meningitis, an emerging zoonosis in intensive pig-rearing countries, caused one fourth (147/617) of adult suspected CNS infection or a half (147/302) of adult purulent bacterial meningitis in Viet Nam. It was reported as an endemic pathogen in all of surveillance regions as well as in the north of Viet Nam (Wertheim et al. 2009). We need more epidemiological studies on risk factors, pathophysiology, treatment and prevention of this disease in both humans and pigs. The overall case fatality rate of CNS infection was 10%. However, we found that case fatality rate of TBM exceeded 30% at the provincial hospitals before TBM diagnosis was established. As patient outcome is directly related to early diagnosis and treatment, physicians should always think of tuberculous meningitis in the context of CNS infection in developing countries.

Chapter 4

Human *Streptococcus suis* serotype 2 meningitis in Viet Nam

4.1 Introduction

Streptococcus suis, a Gram-positive facultatively anaerobic coccus, is a commensal in the respiratory, alimentary and genital tracts of swine. It can cause a wide range of diseases in pigs, such as meningitis, pneumonia, septicaemia and arthritis (Windsor et al. 1975; Gottschalk et al. 2007). According to its capsular antigen, thirty five serotypes isolated from pigs have been described, but serotypes 32 and 34 were recently identified as *Streptococcus orisratti* by biochemical analysis and sequencing (Hill et al. 2005). Among these serotypes, serotype 2 is the most important serotype causing invasive diseases in both human and pig (Staats et al. 1997; Lun et al. 2007; Wertheim et al. 2009). Since the first human case report of *S. suis* infection in Denmark in 1968, increasing numbers of cases have been reported in many European and Asian countries, including the Netherlands, United Kingdom, Hong Kong and Thailand (Arends et al. 1988; Walsh et al. 1992; Kay et al. 1995; Vilaichone et al. 2002). In Viet Nam, alpha-haemolytic streptococci have been reported as a cause of adult bacterial meningitis at Hospital for Tropical Diseases (HTD), since 1995. Since then, the stored isolates were identified as *Streptococcus suis* serotype 2. The number of human cases has increased annually in concurrence with a significant expansion in the scale of the pig farming industry in Viet Nam both in small household and large-scale intensive farming. Around 40% of adults with acute bacterial meningitis patients admitted to HTD have been diagnosed with *S. suis* meningitis since the year 2000 (Mai et al. 2008). Only 30% of *S. suis* meningitis

patients lived in Ho Chi Minh City while the remaining lived in other provinces in southern Viet Nam. However, the pathogen has only been reported at HTD and at another tertiary referral hospital for infectious diseases in Ha Noi (Wertheim et al. 2009) until to date. Whether *S. suis* infection is also commonly found in patients in other hospitals, and thus can be considered a major public health problem in Viet Nam, is unknown. Hence, I conducted a prospective hospital-based surveillance study of *S. suis* infection to determine the incidence rate of *S. suis* infection, at provincial hospitals in central and southern of Viet Nam.

4.2 Aims

1. To describe the epidemiological characteristics of *Streptococcus suis* infection, such as age, sex, occupational exposures to pigs, seasonality, clinical and laboratory manifestations and outcomes, in central and southern Viet Nam.
2. To calculate the incidence rates of *Streptococcus suis* infection in each province and to compare these with the pig density.
3. To describe antimicrobial susceptibility of *Streptococcus suis* strains isolated from human blood and cerebrospinal fluid.

4.3 Materials and Methods

Materials and Methods are presented in the Section 2.3.1, 2.4.2, 2.4.5 and 2.5.

4.4 Results

Between August 2007 and April 2010, we recruited 1645 patients suspected of central nervous system infection at 10 provincial, 2 central and 1 district hospitals in the final analysis, including 774 adult patients and 871 paediatric patients, after excluding 95 patients whose clinical information was unavailable. *S. suis* was identified in 9 of 95 excluded patients (see Section 3.4). We identified 149 *Streptococcus suis* serotype 2 meningitis cases by real-time PCR and culture methods. The bacterium was isolated from CSF and/or blood culture samples of 105 patients. These strains were reported as *Streptococcus* spp, *Streptococcus* group D, alpha or gamma haemolytic *Streptococcus*, *Enterococcus faecalis* or *Enterococcus faecium* in the provincial hospitals. Only at Hue Central hospital, streptococcal isolates were correctly identified as *S. suis*. In this hospital, identification of strains using APIStrep (Biomerieux) was available. CSF and blood cultures of another 44 patients were negative, but specific DNA sequence of *S. suis* serotype 2 was detected in their CSF samples by PCR. Two patients had negative result of PCR of the CSF samples collected after 7-10 days of admission while *S. suis* serotype 2 was isolated from their CSF samples collected on admission.

4.4.1 Characteristics of *Streptococcus suis* meningitis patients

4.4.1.1 Epidemiological characteristics of *S. suis* meningitis patients

S. suis meningitis predominantly occurred in middle-aged male persons. Seventy percent of patients were older than 45 years old and no patient was younger than15 years old. The ratio between males and females was 4:1. Underlying diseases were reported in 26/149 patients, including alcoholism in 25/149 (17%) patients and splenectomy in 1/149 (0.67%) patient. Pig exposures, such as breeding pigs at home, slaughtering pigs and eating undercooked blood/organs, were described in 59/149 (40%) patients. Bacterial meningitis, the most common manifestation of *S. suis* infection, was responsible for 146/149 (98%) cases, and septic shock, the most serious manifestation, was reported in 3/149 cases (2%), all at Hue Central hospital (Table 4-1).

Table 4-1 Epidemiological characteristics of *S. suis* meningitis patients

Characteristics	Patients (n=149)
Age (years), median (IQR)	53 (42; 60)
Age groups (years), n (%)	
- <15	0 (0.00)
- 15 – 29	6 (4.03)
- 30 – 44	38 (25.50)
- 45 – 59	64 (42.95)
- ≥ 60	41 (27.52)
Male sex, n (%)	118 (79.19)
Rural, n (%)	118 (79.19)
Regions, n/x [3] (%)	
- Mekong river delta	75/662 (11.33)
- Central Viet Nam	70/415 (16.87)
- Central Highlands	3/142 (2.11)
- Other (Cambodia and Laos)	1/6 (16.67)
Ethnicity, n (%)	
- Kinh	136 (91.28)
- Khmer	12 (8.05)
- Co Tu	1 (0.67)
Underlying diseases, n (%)	
- Alcoholism	25 (16.78)
- Splenectomy	1 (0.67)

Pig exposures, n (%)	
- Pigs at home	37 (24.83)
- Exposures to pigs or pork[1]	36 (24.16)
- Eating undercooked pig blood or organs[2]	26 (17.45)
- At least one of three above factors	59 (39.60)
Diagnosis, n (%)	
- Bacterial meningitis	146 (97.99)
- Septic shock	3 (2.01)
Duration of hospital stay (days), median (IQR)	19 (14; 23)

[1] Bathing, feeding and slaughtering pigs; cleaning up the piggery and preparing or handling blood, organs from pigs

[2] Fresh/undercooked blood, tonsils, tongue, stomach, intestines and uterus.

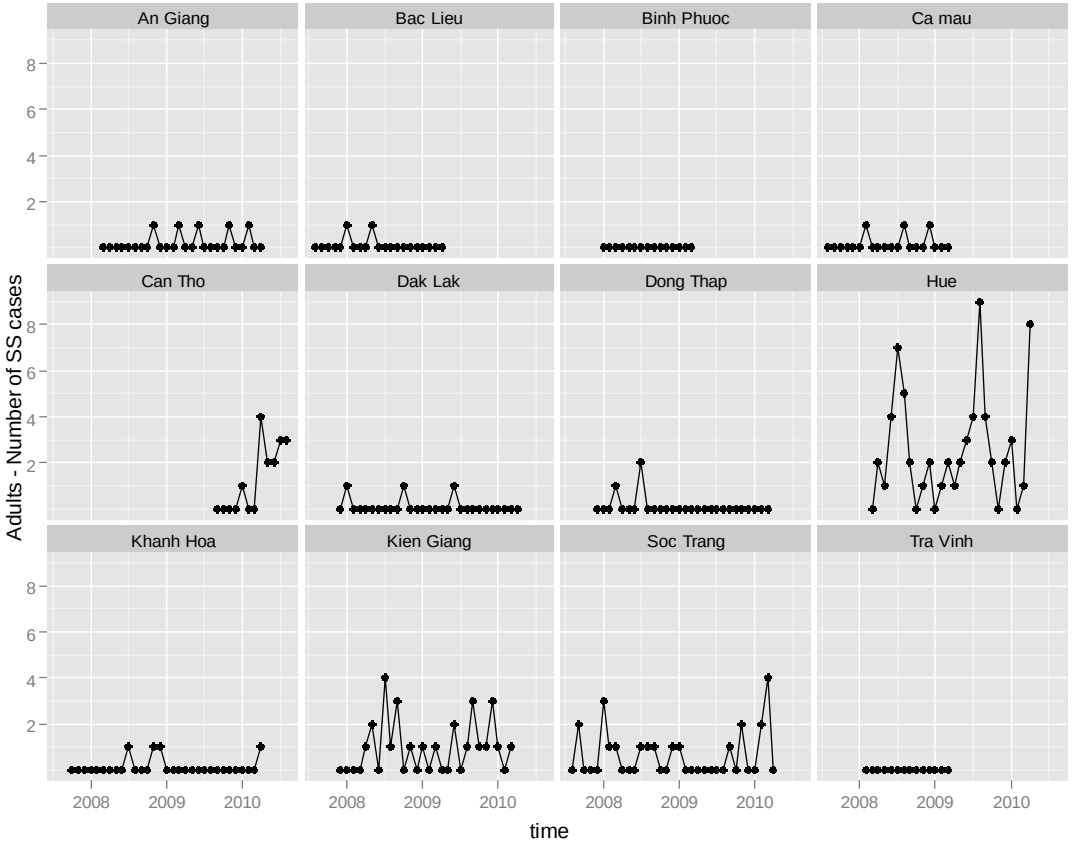
[3] x is the number of patients recruited in each region.

4.4.1.2 Seasonality of *S. suis* meningitis

The admission characteristics of *S. suis* meningitis cases followed a seasonal distribution ($p<0.001$), of which the peak month was estimated as July and the amplitude of difference (peak month vs. average) was + 70% (95%CI [+34%; 116%]). A linear time trend analysis of infection showed an increase of 38% per year (95%CI [6%; 81%], $p=0.018$) which remained significant after adjustment for overdispersion ($p=0.033$). However, seasonality seemed to be driven by the admission characteristics of Hue Central hospital only. In an analysis for each of the hospitals, the test for seasonality was significant for Hue hospital ($p<0.001$) while it was not significant for all other hospitals ($p=0.31$). A linear time trend analysis of infection in Hue, showed an increase of 73% per year (95%CI [12%; 170%], $p=0.013$) and which

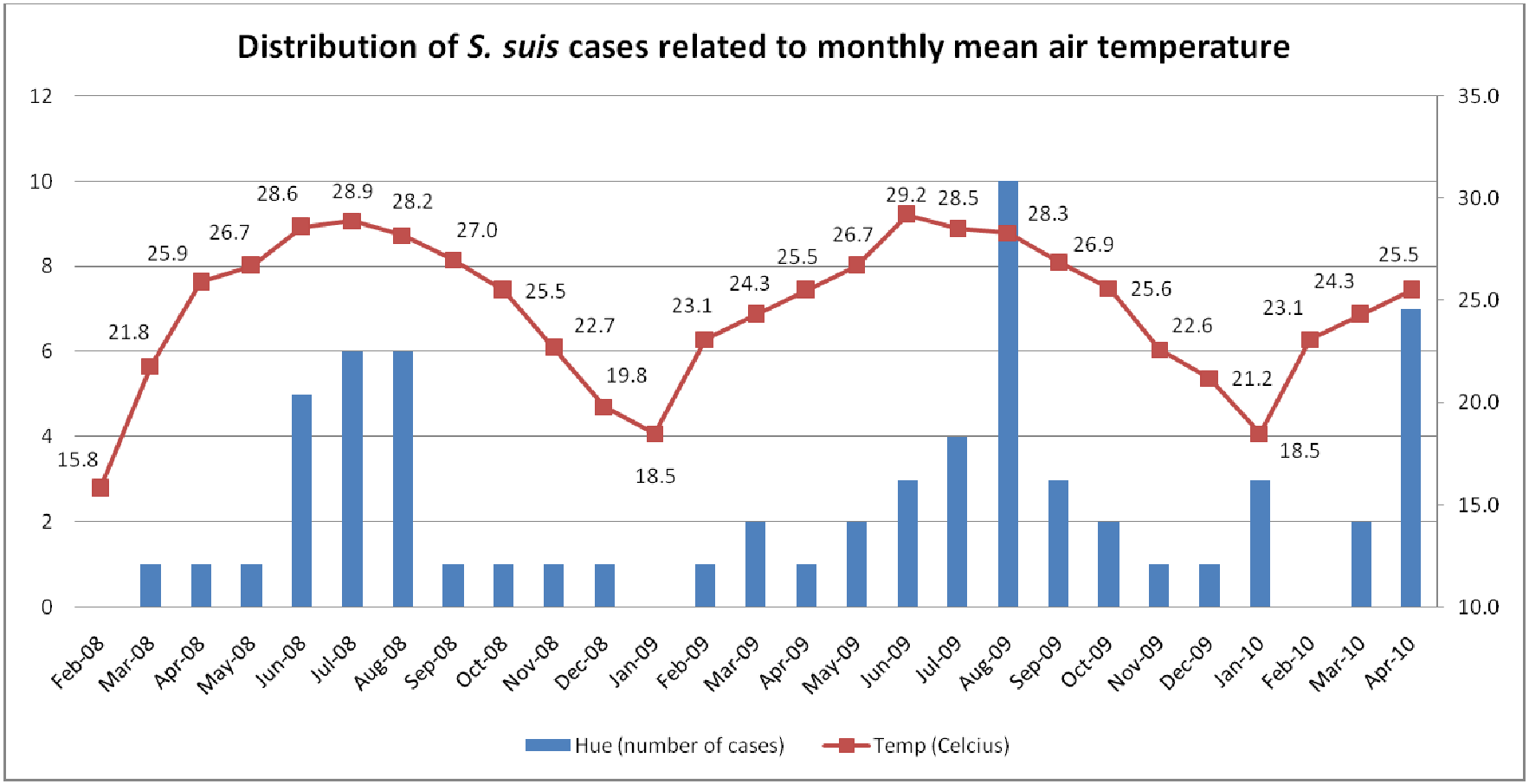
remained significant after adjustment for overdispersion (p=0.033). Such trend was not observed for other hospitals than Hue (p=0.15) (Figure 4-1).

Figure 4-1 Time distribution of *S. suis* meningitis admission by hospitals



Admission of *S. suis* patients coincided with high air temperature for hospitals in North Central Viet Nam (Figure 4-2).

Figure 4-2 Admission of *S. suis* meningitis and monthly mean air temperature in Hue Central hospital



4.4.1.3 Clinical and laboratory characteristics of *S. suis* meningitis

Patients with *S. suis* meningitis presented within a median of 3 days of illness Over 90% patients had typical manifestations of meningitis, such as fever, headache, vomiting and meningeal signs. Ninety eight of 149 patients (66%) were confused on admission. Hemorrhagic rash, similar to the rash of meningococcemia, was reported in 6/149 (4%) patients. The white cell count of the cerebrospinal fluid, which was cloudy in 70% of patients, was greater than 240/μl in 108/145 (75%) of patients and the percentage of neutrophiles was greater than 60% in 94/124 (76%) of patients. High CSF protein and low CSF glucose were recorded in most of patients. Although 67/149 (45%) of patients had received antibiotics prior to admission, CSF and/or blood culture were positive in 105/149 (70%) patients. For the remaining 30% of patients, *S. suis* infection was confirmed by real-time PCR method (Table 4-2).

Overall case fatality rate (CFR) was 8% (12/149), but CFR of septic shock cases reached 100% (3/3), compared to 9/146 (6%) of meningitis cases. Septic shock patients were admitted in 2 or 3 days of illness and none of them received antimicrobial treatment prior to admission. Pig exposure was only reported in one patient who reported to breed pigs, and who also suffered from alcoholism. Eating fresh blood or undercooked pig intestines was not documented in these patients. All of them had hemorrhagic rash, altered consciousness and meningitis syndrome. *S. suis* serotype 2 was isolated from CSF samples of 3/3 patients and from blood samples of 2/3 patients. In addition, 46/149 (31%) patients suffered hearing impairment at discharge, of which deafness was recorded in 18/149 (12%) patients. Patients with hearing impairment were older than patients without this sequela, 55 years of age (median) compared to 50 years of age, respectively (p= 0.012) (Table 4-3).

Table 4-2 Clinical and laboratory characteristics of *S. suis* meningitis patients

Characteristics	Adult patients (n=149)
Duration of illness (days), median (IQR)	3 (2; 4)
Clinical manifestations, n (%)	
- Fever	145 (97.32)
- Headache	139 (93.29)
- Nausea/Vomiting	107 (71.81)
- Neck stiffness	130 (87.25)
- Diarrhea	8 (5.37)
- Tinnitus	46 (30.87)
- Deafness	18 (12.08)
- Altered consciousness	98 (65.77)
- Convulsion	18 (12.08)
- Hemiplegia	6 (4.03)
- Cranial nerve palsy	4 (2.68)
- Herpes labialis	9 (6.04)
- Hemorrhagic rash	6 (4.03)
Laboratory investigations, median (IQR)	
- White blood cells	15400 (11200; 20400)
o Percentage of neutrophiles	88 (81.5; 91.6)
o Percentage of lymphocytes	7 (4.3; 10.4)
- Platelets	159500 (106000; 206500)

- Cerebrospinal fluid	
o Cloudy, n (%)	106 (71.14)
o White cells	882 (240; 3100)
o Percentage of neutrophiles	85 (63.5; 90)
o Protein (g/l)	2.15 (1.2; 3.83)
o Glucose (mmol/l)	0.9 (0.3; 2)
o Ratio of CSF and blood glucose	0.11 (0.04; 0.25)
- CSF Gram stain positive, n (%)	43 (28.86)
- CSF culture positive, n (%)	102 (68.46)
- CSF PCR positive, n/N (%)	114/116 (98.28)
- Blood culture positive, n/x (%)	33/90 (36.67)
No antibiotics prior to admission, n (%)	82 (55.03)
Outcomes, n (%)	
- Alive	127 (85.23)
- Died	12 (8.05)
- Transferred to other hospital	10 (6.71)

Table 4-3 Comparison of characteristics of patients with and without hearing impairment[1]

Characteristics	Hearing impairment (n= 46)	Normal hearing (n= 83)	p value [2]
Duration of illness (days), median (IQR)	3 (2; 5)	3 (2; 4)	0.207
No antibiotics prior admission, n (%)	25 (54.35)	49 (59.04)	0.606
Age (years), median (IQR)	55 (46; 63)	50 (41; 58)	0.012
Male sex, n (%)	34 (73.91)	67 (80.72)	0.369
Kinh ethnicity, n (%)	41 (89.13)	78 (93.98)	0.327
Pig exposures[3], n (%)	14 (30.43)	15 (18.07)	0.107
Eating habit[4], n (%)	6 (13.04)	15 (18.07)	0.459

[1] Twenty cases with missing data on hearing impairment were excluded.

[2] Using Chi-square test or Fisher's exact test (when one or more of the expected count is less than 5) for categorical variables and Wilcoxon rank sum test for continuous variables.

[3] Pig breeder, butcher, and abattoir worker

[4] Eating fresh blood, pig intestines and uterus.

4.4.2 Incidence rate of *Streptococcus suis* serotype 2 meningitis

The overall incidence rate of *S. suis* serotype 2 meningitis was 0.57 per 100,000 adult person-years. The incidence rate was highest in Thua Thien – Hue province with 3.32 per 100,000 adult person-years. It also increased significantly with incremental age group. Incidence rate in age group 60+ was 30 times higher than in age group 15-29 (1.66 compared to 0.05 per 100,000 person-years) (Table 4-4, 4-5, 4-6 and 4-7).

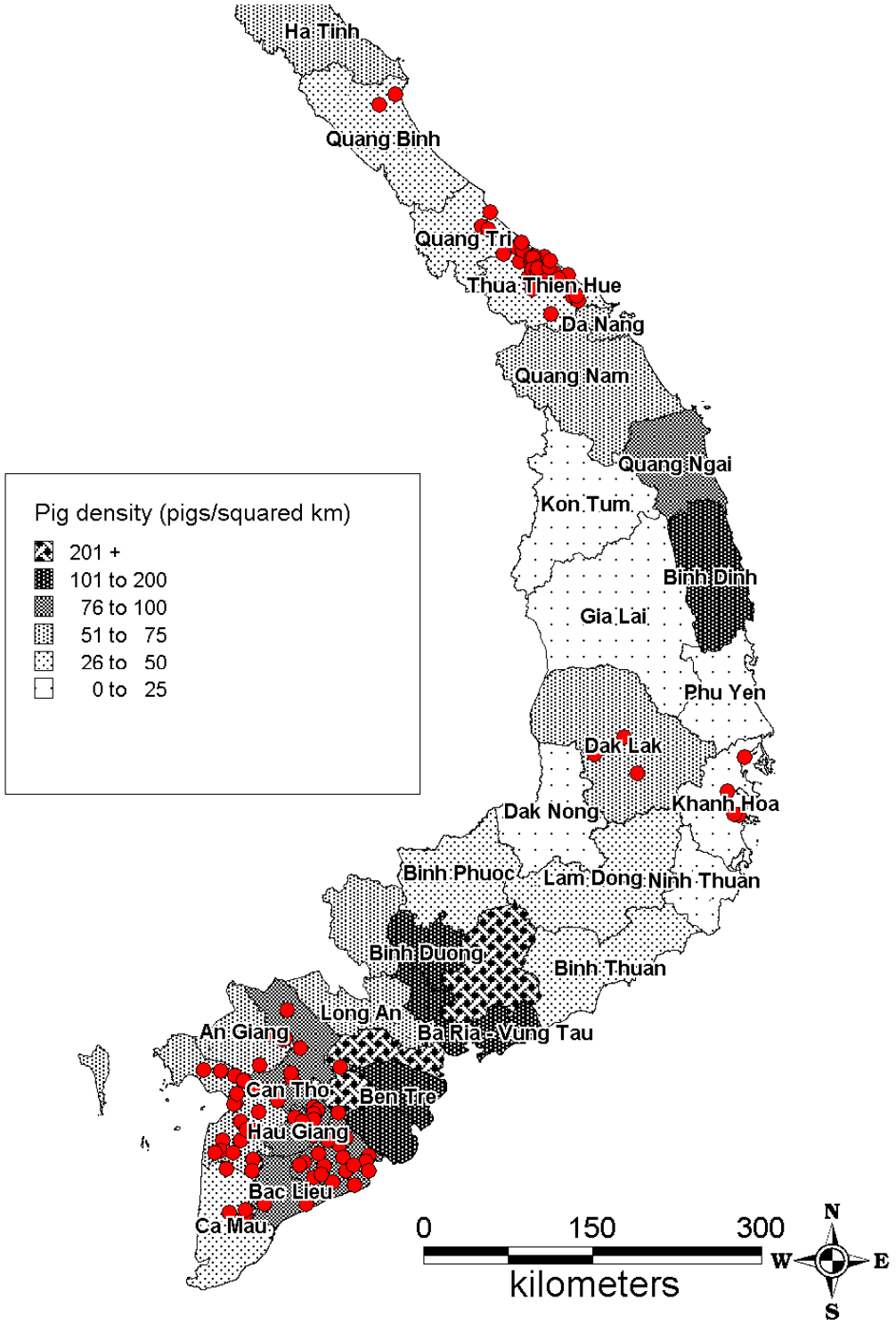
In addition, this study addressed whether the incidence of *S. suis* infection could be related to the development of pig rearing industry in the community. Figure 4-3 depicts the distribution of *S. suis* meningitis cases and the corresponding provincial pig density on the Viet Nam map. Figure 4-4 shows the incidence rate per province and the corresponding provincial pig density. Mapping did not show a clear association between a high pig density and incidence rate of *S. suis* infection. I did not find any evidence for an association of the incidence rate of human *S. suis* infection and pig density (p=0.62). Maybe, pig density varies by month but I do not have this information, except the mean pig density in a whole year.

Table 4-4 The incidence rates of *Streptococcus suis* serotype 2 meningitis in adult population by regions

Regions	Total person-time of observation ($\times 10^5$ person-years)	Adult population (≥ 15 years of ages)		
		Proportion of adult group[1]	Number of patients	Incidence rate (95%CI)
Mekong river delta	**161.324**	**0.76**	**48**	**0.39 (0.29; 0.52)**
- Ca Mau	12.017	0.75	3	0.33 (0.07; 0.97)
- Bac Lieu	8.475	0.76	1	0.16 (0.00; 0.87)
- Soc Trang	25.783	0.75	10	0.52 (0.25; 0.95)
- Can Tho	11.896	0.78	4	0.43 (0.12; 1.10)
- Tra Vinh	10.008	0.77	0	-
- Dong Thap	16.625	0.76	2	0.16 (0.02; 0.57)
- An Giang	42.918	0.76	5	0.15 (0.05; 0.36)
- Kien Giang	33.602	0.74	23	0.92 (0.59; 1.39)
Central Viet Nam	**44.826**	**0.73**	**55**	**1.68 (1.27; 2.19)**
- Khanh Hoa	23.090	0.74	3	0.18 (0.04; 0.51)
- Thua Thien-Hue	21.736	0.72	52	3.32 (2.48; 4.36)
Central Highlands				
- Dak Lak	34.482	0.69	3	0.13 (0.03; 0.37)
South East				
- Binh Phuoc	8.580	0.71	0	-
Overall	**249.212**	**0.74**	**106**	**0.57 (0.47; 0.70)**

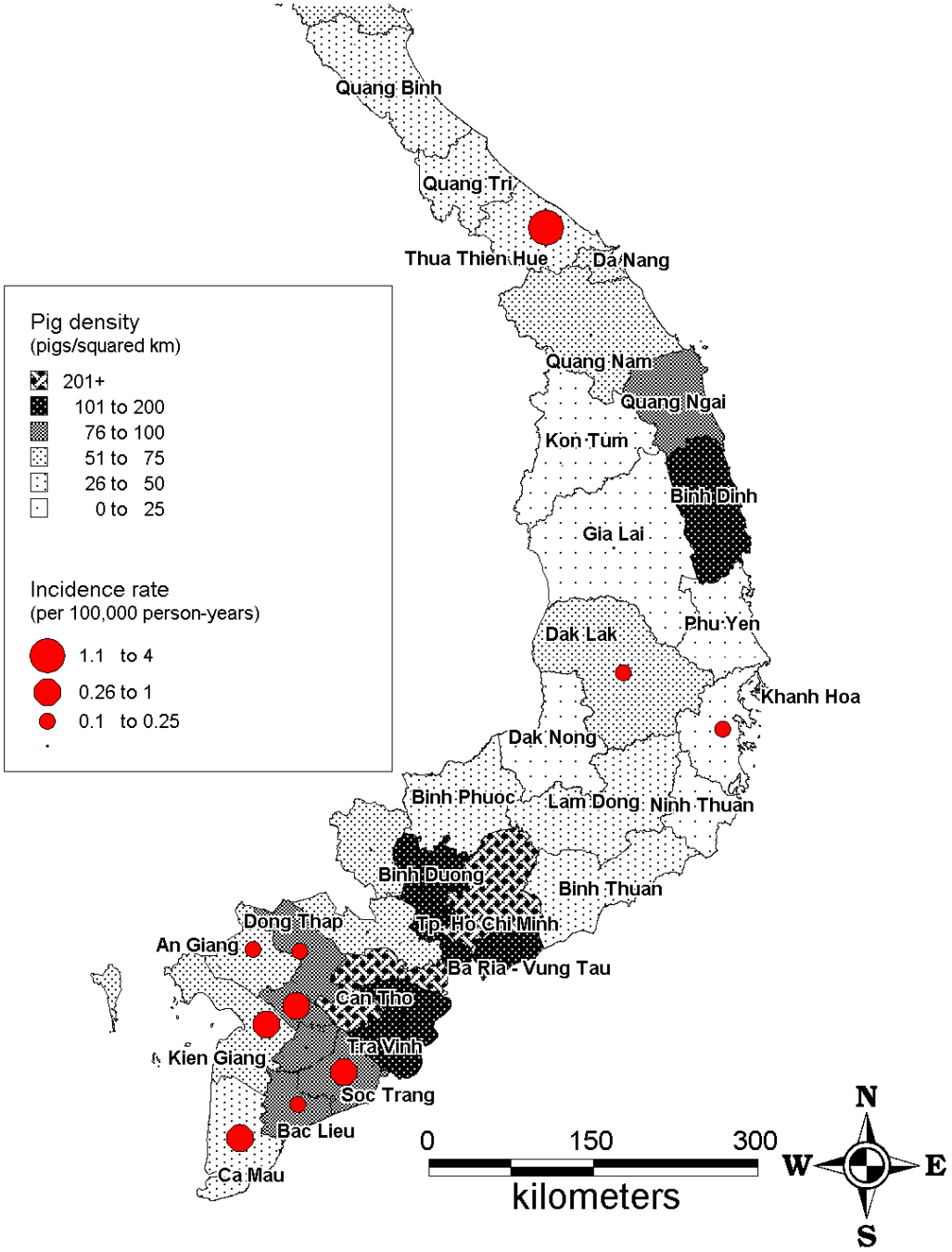
[1] Using data of population by age group from the 2009 Vietnam population and house census (Table 2-2) (CP&HCSC 2010)

Figure 4-3 Map of human *S. suis* cases with background pig density data[1]



[1] Pig density was retrieved from Statistical Yearbook of Viet Nam 2009 (GSO 2010) and each red dot represents one human *S. suis* case.

Figure 4-4 Map of incidence rate of *S. suis* infection per province with background pig density data[1]



[1] Pig density was retrieved from Statistical Yearbook of Viet Nam 2009 (GSO 2010)

Table 4-5 Incidence rate of *Streptococcus suis* serotype 2 meningitis by age groups and regions (A)

Regions	Total person-time of observation ($\times 10^5$ person-years)	Age group (years)					
		15-29			30-44		
		Proportion of age group[5]	Number of patients	Incidence rate (95%CI)	Proportion of age group[5]	Number of patients	Incidence rate (95%CI)
Mekong river delta[1]	161.324	0.30	2	0.04 (0.01; 0.15)	0.24	17	0.44 (0.26; 0.70)
Central Viet Nam[2]	44.826	0.28	2	0.16 (0.02; 0.58)	0.23	8	0.78 (0.33; 1.53)
Central Highlands[3]	34.482	0.29	0	-	0.22	0	-
South East[4]	8.580	0.30	0	-	0.23	0	-
Overall	249.212	0.30	4	0.05 (0.01; 0.14)	0.23	25	0.44 (0.28; 0.64)

[1] Ca Mau, Bac Lieu, Soc Trang, Can Tho, Tra Vinh, Dong Thap, An Giang and Kien Giang.

[2] Thua Thien – Hue and Khanh Hoa.

[3] Dak Lak

[4] Binh Phuoc

[5] Applying data of population by age group in the 2009 Vietnam population and house census (Table 2-2) (CP&HCSC 2010)

Table 4-6 Incidence rate of *Streptococcus suis* serotype 2 meningitis by age groups and regions (B)

Regions	Total person-time of observation ($\times 10^5$ person-years)	Age group (years)					
		45-59			60+		
		Proportion of age group[5]	Number of patients	Incidence rate (95%CI)	Proportion of age group[5]	Number of patients	Incidence rate (95%CI)
Mekong river delta[1]	161.324	0.14	19	0.84 (0.51; 1.31)	0.08	10	0.77 (0.37; 1.42)
Central Viet Nam[2]	44.826	0.14	23	3.66 (2.32; 5.50)	0.09	22	5.45 (3.42; 8.26)
Central Highlands[3]	34.482	0.12	2	0.48 (0.06; 1.75)	0.06	1	0.48 (0.01; 2.69)
South East[4]	8.580	0.13	0	-	0.06	0	-
Overall	249.212	0.14	44	1.26 (0.92; 1.69)	0.08	33	1.66 (1.14; 2.32)

[1] Ca Mau, Bac Lieu, Soc Trang, Can Tho, Tra Vinh, Dong Thap, An Giang and Kien Giang.

[2] Thua Thien – Hue and Khanh Hoa.

[3] Dak Lak

[4] Binh Phuoc

[5] Applying data of population by age group in the 2009 Vietnam population and house census (Table 2-2) (CP&HCSC 2010)

Table 4-7 Comparison of incidence rates of *S. suis* meningitis across age groups

Overall incidence rate	Incidence Rate Ratio (IRR)	95% CI of IRR	p value
15-29	1	-	-
30-44 vs. 15-29	8.15	[2.84; 23.42]	<0.001
45-59 vs. 15-29	23.57	[8.47; 65.60]	<0.001
60+ vs. 15-29	30.94	[10.96; 87.33]	<0.001

4.4.3 Antimicrobial susceptibility of *Streptococcus suis* serotype 2

We received 86/105 *Streptococcus suis* serotype 2 strains from provincial hospitals. *S. suis* was completely sensitive to penicillin, ceftriaxone, cefepime, levofloxacin and vancomycin (Table 4-8).

Table 4-8 Antimicrobial susceptibility of *S. suis* serotype 2 strains isolated from meningitis patients

Antimicrobial agent (number of isolates tested)	Minimal Inhibitory Concentrations (μg/ml)				Resistant strains, n (%)
	Breakpoints[1]	Range	MIC_{50} [2]	MIC_{90} [2]	
Penicillin (n=86)	S ≤ 0.12	0.016 to 0.094	0.047	0.064	0 (0.00)
Ceftriaxone (n=86)	S ≤ 1; R ≥ 4	0.032 to 0.25	0.094	0.125	0 (0.00)
Cefepime (n=86)	S ≤ 1; R ≥ 4	0.016 to 0.094	0.047	0.094	0 (0.00)
Levofloxacin (n=86)	S ≤ 2; R ≥ 8	0.19 to 1.00	0.38	0.38	0 (0.00)
Vancomycin (n=85)	S ≤ 1	0.19 to 0.75	0.25	0.50	0 (0.00)
Chloramphenicol (n=86)	S ≤ 4; R ≥ 16	2 to 64	3	4	4 (4.65)
Tetracycline (n=86)	S ≤ 2; R ≥ 8	1.5 to >256	24	32	81 (94.19)
Erythromycin (n=86)	S ≤ 0.25; R ≥ 1	0.023 to >256	0.064	>256	13 (15.12)

[1] Breakpoints are based on equivalent MIC breakpoints for *Streptococcus spp.* viridians group (CLSI 2010). R and S mean resistant and sensitive.

[2] MIC_{50} and MIC_{90} are the MIC values inhibiting growth of 50% and 90% of tested isolates, respectively

4.5 Discussion

4.5.1 Epidemiological characteristics of *Streptococcus suis* meningitis

Though *Streptococcus suis* is a rare cause of purulent bacterial meningitis in European and North America countries, it is the second most common pathogen in Hong Kong and the most common pathogen in Viet Nam (Hui et al. 2005; Mai et al. 2008; Wertheim et al. 2009). The overall incidence rate in Vietnamese adult population was 0.57 per 100,000 person-years. Assuming that 25% of *S. suis* patients had pig or pork exposure factor (Table 4-1), the estimated incidence rate in the adult population without any exposure to pig or pork was 0.43/100,000 person-years (95% CI [0.34; 0.54] per 100,000 person-years), compared to 0.09/100,000 and 0.002/100,000 in Hong Kong and the Netherlands, respectively (Arends et al. 1988; Ma et al. 2008). It was more than 5 times that of Hong Kong and 200 times that of the Netherlands. This data and other previous case series studies in Viet Nam indicated that *Streptococcus suis* serotype 2 infection is endemic in Viet Nam (Mai et al. 2008; Wertheim et al. 2009). No *S. suis* meningitis cases were detected in 871 children presenting with central nervous system infection. Only one case of *S. suis* meningitis has been reported in a Thai child indicating that this infection is extremely rare among children (Vilaichone et al. 2002; Mai et al. 2008). The incidence rate increased with incremental age in the adult population (Table 4-7) and 75% of patients were older than 45 years of age. A higher rate of disease in the elderly was also reported in other studies (Wertheim et al. 2009). This observation could be explained by more frequent or more intensive exposure to pigs or pork among elderly people compared with

children and younger adult, or by decreased immune status and/or a higher rates of underlying disease with increase of age (Ma et al. 2008).

Similar to other case series, *S. suis* infection occurred predominantly in male sex. The high male-to-female ratio could be explained by the fact that this infection is a predominantly an occupational disease (Arends et al. 1988). However, occupational exposure to pig or pork was only reported in 24% (36/149) of patients, compared to 33% (50/151) of another study in Viet Nam and 24% (5/21) of a study in Hong Kong (Ma et al. 2008; Mai et al. 2008). While occupational exposure was recorded less than 40% in Asian studies, it was reported in nearly 90% of European cases (Kay et al. 1995). This difference could be explained by the underreporting of this risk factor for Asian patients, or by the presence of other behavioural or exposure related risk factors in the Asian population, such as culinary habits or proximity of pigs within households. Mapping of cases and comparison with pig density in each province showed that the risk of *S. suis* infection may be unrelated to pig density in a region. Seasonality of *S. suis* meningitis was not observed in southern provinces. This result was similar to a previous report of a large case series at Hospital for Tropical Diseases in Ho Chi Minh City (Mai et al. 2008). However, more cases and outbreaks were reported in the hottest months of the year in reports from northern Thailand, northern Viet Nam and China (Kay et al. 1995; Wangkaew et al. 2006; Yu et al. 2006; Ma et al. 2008; Wertheim et al. 2009). Similarly, seasonality of *S. suis* meningitis was also reported in our study at Hue Central hospital, a tertiary hospital of the North Central of Viet Nam. The difference between the Southern and the Northern provinces could be explained by the large fluctuation of air temperature in the Northern provinces. Human *S. suis* infection tended to occur in parallel with pig diseases in the hottest

months of the summer in China (Ma et al. 2008). Stresses, such as crowding, poor ventilation, and sudden weather change, predispose pigs to *S. suis* serotype 2 infection (Staats et al. 1997). Moreover, high bacterial load in meat sold at the wet markets without being preserved in fridges may be transmitted to human in the summer. Hence, pig carriage or contamination of meat in slaughter houses may be a risk at higher temperature.

4.5.2 Clinical manifestations and outcome of *S. suis* serotype 2 meningitis

Skin rash usually presents in septic shock syndrome, a life-threatening manifestation of *S. suis* infection, in which case fatality rate exceeded 60% (38/61) in Sichuan outbreak, compared to 1% (1/102) of meningitis (Yu et al. 2006). This rash is similar to the rash of meningococcemia. The rash of meningococcemia usually occurs in children or young adult while that of *S. suis* infection often present in middle-aged patients. Incubation time of septic shock cases was shorter than that of meningitis cases in the Sichuan outbreak, 1.6 days compared to 2.2 days respectively ($p<0.05$) (Yu et al. 2006). The case fatality rate in these studies from provincial hospitals was higher than that reported from the Hospital for Tropical Diseases a tertiary referral hospital in Ho Chi Minh City (8% vs. 2.6%) (Mai et al. 2008). The reason may be related to the high mortality rate of septic shock in this study compared to that of meningitis (100% (3/3) vs. 6.2% (9/146)). Septic shock was also an independent risk factor for mortality in a Thai study, where 6/8 (75%) septic shock patients died and hazard ratio was 22.03 (95% CI, 1.88-158.28) (Wangsomboonsiri et al. 2008). The reasons of development of septic shock may be delay of admission or starting antimicrobial therapy, high virulence of pathogen and factors related to host status, such as decreased immune status and underlying diseases. Fulminant meningococcal

septicaemia is characterized by a rapid proliferation of meningococci in the circulation, resulting in very high concentration of bacteria (10^5–10^8/ml) and meningococcal endotoxin (10^1–10^3 endotoxin units/ml)in blood, compared to low concentration of meningococci (<10^3/ml) and endotoxin (< 3 endotoxin units/ml) in plasma of meningitis patients (Stephens et al. 2007).

Apart from the high rate of hearing loss, signs and symptoms of *S. suis* meningitis could not be differentiated from purulent bacterial meningitis caused by other bacteria (Table 4-9). Hearing loss ranged from 30% to 90% in different studies depending on the proportion of meningitis cases in the study sample (Walsh et al. 1992; Mai et al. 2008; Rusmeechan et al. 2008). In a series of 41 cases from Chiang Mai University hospital, 30.7% (4/13) of meningitis cases and none of 16 infective endocarditis cases developed hearing loss (Wangkaew et al. 2006). Animal experiments demonstrated suppurative labyrinthitis, with the bacteria invading the perilymph via the cochlear aqueduct (Kay 1991). This hypothesis was indirectly confirmed in human *S. suis* meningitis cases by computed tomography and magnetic resonance imaging scans of the inner ears of a Thai patient (Navacharoen et al. 2009). In summary, *S. suis* meningitis should be suspected in any Vietnamese adult with bacterial meningitis regardless of occupational exposure to pigs or pork.

4.5.3 Antimicrobial susceptibility of *Streptococcus suis* serotype 2 strains

Streptococcus suis serotype 2 strains isolated from humans are generally susceptible to antimicrobial agents used to treat purulent bacterial meningitis, such as penicillin, ampicillin, ceftriaxone and vancomycin (Wangkaew et al. 2006; Ma et al. 2008; Mai et al. 2008; Hoa et al. 2011). However, one strain isolated from a Thai patient with peritonitis was reported as penicillin resistant with MIC>32 μg/ml

(Vilaichone et al. 2002). The antimicrobial susceptibility rates of our strains collected in the period 2008-2010, were similar to the rates observed in the strains collected at Hospital for Tropical Diseases in 2004-2008, except that the chloramphenicol resistance rate was lower in our study (4.65% vs.13%) (Hoa et al. 2011). The use of chloramphenicol in agriculture has been banned in Vietnam since 2003. However, other amphenicols (such as florfenicol) are still allowed to use in agriculture and animal husbandry in Vietnam. It is also possible that the chloramphenicol resistance determinants are under co-selection in strains that are resistant to tetracyclines and macrolides, which currently approved for veterinary use in the prevention and treatment of infections (Hoa et al. 2011).

Table 4-9 Clinical manifestations and outcome of *S.suis* infection

Characteristics	This study (n=149)	HTD, Viet Nam[1] (n=151)	NHTD, Viet Nam[2] (n=50)	Thailand[3] (n=41)	UK[4] (n=35)
Duration of illness (days), median (IQR)	3 (2; 4)	4 (3;5)	4	-	-
Clinical manifestations, n (%)					
- Fever	145 (97.32)	148 (98.01)	50 (100.00)	39 (95.12)	17/20 (85.00)
- Headache	139 (93.29)	142 (94.04)	46 (92.00)	40 (97.56)	-
- Nausea/Vomiting	107 (71.81)	100 (66.23)	-	-	-
- Neck stiffness	130 (87.25)	142 (94.04)	44 (88.00)	39 (95.12)	17/20 (85.00)
- Diarrhea	8 (5.37)	9 (5.96)	-	-	-
- Hearing loss	46 (30.87)	93/140 (66.43)	16 (32.00)	38 (92.68)	10/28 (35.71)
- Altered consciousness	98 (65.77)	104 (68.87)	23 (46.00)	22 (53.66)	-
- Hemiplegia	6 (4.03)	15 (9.93)	-	1 (2.44)	-
- Cranial nerve palsy	4 (2.68)	13 (8.61)	-	2 (4.88)	-
- Hemorrhagic skin rash	4 (2.68)	9 (5.96)	7 (14.00)	0 (0)	-
Laboratory investigations, median					
- White blood cells	15,400	16,800	17,900[5]	-	-

- Platelets	159,500	159,000	169,800[5]	-	-
- Cerebrospinal fluid					
o White cells	882	2,100	3,253[5]	-	-
o Percentage neutrophiles	85	84	-	-	-
o Protein (g/l)	2.15	2.06	1.70[5]	-	-
o Ratio of CSF and blood glucose	0.11	0.14	-	-	-
- CSF culture positive, n (%)	102 (68.46)	115 (76.16)	32(64.00)	37 (90.24)	-
Diagnosis					
- Bacterial meningitis, n (%)	146 (97.99)	149 (98.68)	44 (88.00)	41 (100.00)	18/20 (90.00)
- Septic shock, n (%)	3 (2.01)	2 (1.32)	6 (12.00)	-	-
Died, n(%)	12 (8.05)	4 (2.65)	3 (6.00)	0 (0)	4 (11.43)

[1] (Mai et al. 2008)

[2] (Wertheim et al. 2009)

[3] (Rusmeechan et al. 2008)

[4] (Walsh et al. 1992)

[5] Mean was presented

4.5.4 Limitation of study

Though most human *Streptococcus suis* infection cases are caused by *S. suis* serotype 2, other serotypes were also reported as a pathogen of meningitis or sepsis, such as serotype 4 (Arends et al. 1988), serotype 14 (Mai et al. 2008; Haleis et al. 2009; Kerdsin et al. 2009) and serotype 16 (Nghia et al. 2008). Real-time PCR test is a sensitive tool in surveillance of *S. suis* meningitis, especially when antibiotics had been used prior to the withdrawal of CSF sample (Mai et al. 2008). However, the PCR test targeted a specific DNA sequence of serotype 2 and this would miss patients infected with other serotypes.

Both classical microbiological culture and diagnostic PCR was used for detecting *S. suis* cases, but the incidence rate could be still underestimated for a variety of reasons, including deaths prior to admission, primary presentation other than meningitis, e.g. sepsis syndrome, or because of the presence of a contraindication to lumbar puncture procedure.

Finally, there was not data on the number of people who are pig breeders, abattoir workers or butchers in Viet Nam. Therefore, it was not possible to calculate the incidence rates of *S. suis* infection for these important groups.

4.6 Conclusions

This study was the first prospective surveillance study of *Streptococus suis* meningitis in the Southeast Asia area. *S. suis* serotype 2 meningitis cases were reported in most of surveillance provinces in central and southern Viet Nam. Seasonality of this infection was documented in the North Central of Viet Nam, but not in southern Viet Nam. The overall incidence rate in adult population was 0.57 per 100,000 person-years. The estimated incidence rate among the general adult population was more than 5 times that of Hong Kong and 200 times that of the Netherlands.

S. suis infection is a striking public health problem in Viet Nam associated with substantial mortality and morbidity. Occupational exposure to pig or pork was only recorded in 25% of patients. Hence, there was a need to conduct an epidemiological study to identify additional risk factors in order to advise on preventative measures for *Streptococcus suis* infection in Viet Nam.

Chapter 5

Risk factors for *Streptococcus suis* infection in Viet Nam

5.1 Introduction

The importance of zoonotic emerging infections is increasingly recognized, as illustrated by outbreaks of SARS coronavirus and the ongoing threat of human infections with avian influenza H5N1 virus. It is essential to understand transmission dynamics and risk factors for infection, in order to design effective strategies to contain and prevent the spread of zoonotic diseases (Ma et al. 2009). *Streptococcus suis* infection is an emerging zoonotic infectious disease, which is increasingly reported in Southeast Asia and China. In Viet Nam, *S. suis* infection was first reported in 1996 and the number of human cases has increased annually, coinciding with a significant expansion in the scale of the pig farming industry in Viet Nam both in small household and large scale intensive farming. Among approximately 100 adult patients admitted with acute bacterial meningitis to the Hospital for Tropical Diseases Ho Chi Minh City each year, around 40% will be diagnosed with *S. suis* meningitis, which is more than *Streptococcus pneumoniae* and *Neisseria meningitidis* combined. Thus, the disease can be considered endemic in Viet Nam (Nguyen et al. 2007; Mai et al. 2008; Wertheim et al. 2009). To date little is known of the risk factors underlying the disease or the portals of entry in humans. From small case series, the reported risk factor included occupational exposure, such as in slaughter house workers, butchers, pig breeders, meat processing and pig transportation workers. It is hypothesized that patients may be infected through minor cuts or abrasions on their skin (Arends et al. 1988; Yu et al. 2006). However, although an occupational exposure to pigs or pork

was documented in 88% of the European patients described, it was reported in only up to 42% of the Asian cases (Kay et al. 1995; Mai et al. 2008). I hypothesised that there may be other behavioural or exposure related risk factors in Asian populations, such as culinary habits, or close proximity of pigs within households, and more intensive exposure to pigs or pork meat in the Asian context than is usual in European populations. Hence I conducted a prospective case-control study to identify the risk factors of *S. suis* infection in Viet Nam.

5.2 Aims

To identify risk factors of *Streptococcus suis* infection in Vietnam

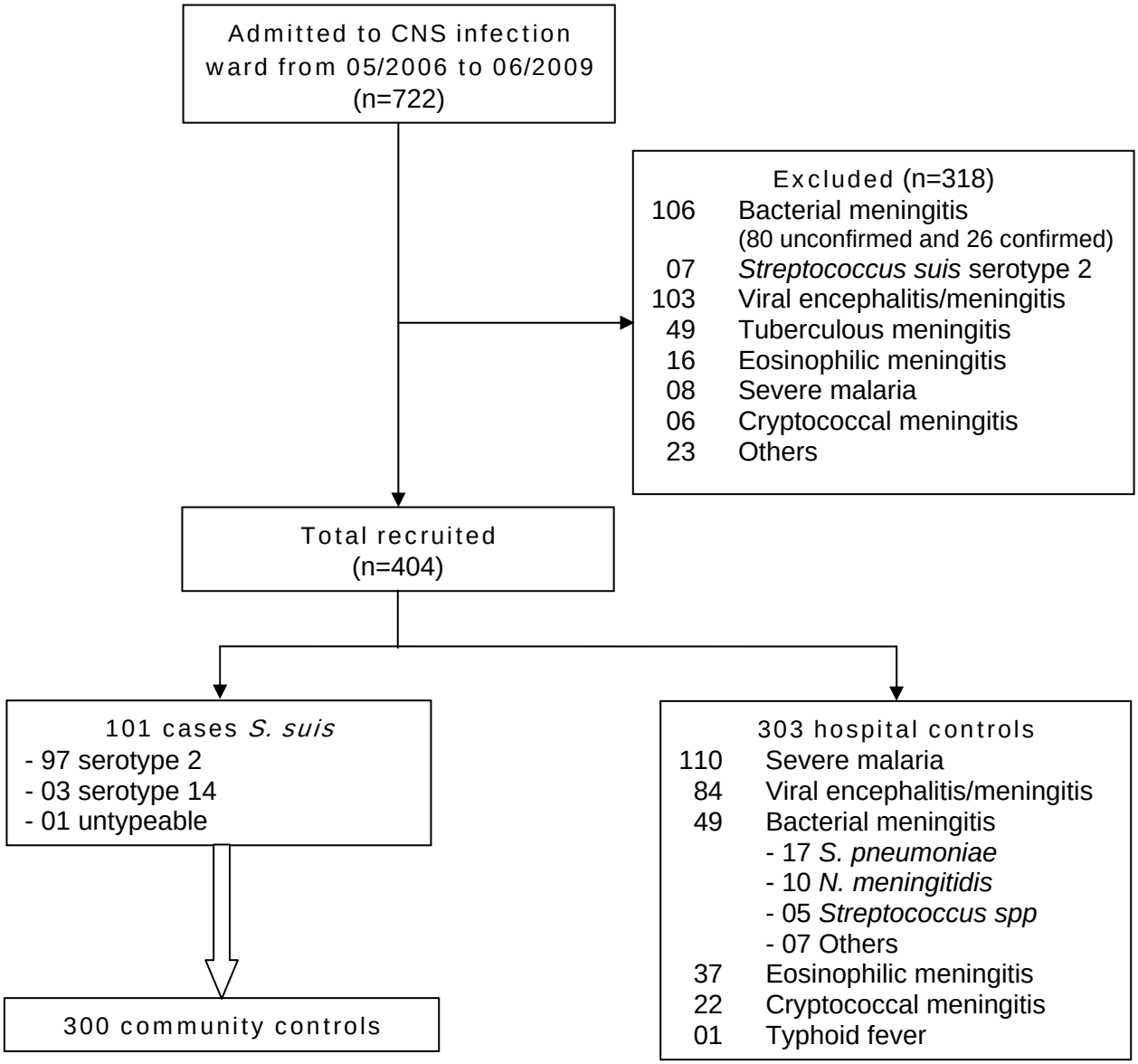
5.3 Materials and Methods

Materials and Methods are described in Section 2.3.2, 2.4.2, 2.4.3.2, 2.4.4, 2.4.5 and 2.5

5.4 Results

Between May 2006 and June 2009, a total of 722 patients with suspected CNS infections or severe malaria were admitted to HTD. *S. suis* meningitis was diagnosed in 108 patients. Seven cases were excluded as they did not meet the inclusion criteria (HIV positive in 3 cases and prolonged coma over 14 days in 1 case), were diagnosed as tuberculous meningitis (2 cases), or were unable to communicate due to language differences (one Cambodian case). Another 311 patients, including 106 non-*S. suis* bacterial meningitis patients, 103 viral encephalitis/meningitis patients, 49 tuberculous meningitis patients, 16 eosinophilic meningitis patients, 6 cryptococcal meningitis patients, 8 severe malaria patients and 23 other patients, were excluded because of death (37 cases), prolonged coma (72 cases), unconfirmed bacterial meningitis (80 cases), transfer to other hospitals (61 cases), use of antimicrobial agents more than 2 days in case of suspected viral encephalitis/meningitis (38 cases), and absence of diagnosis of CNS infection (23 cases). It was not possible to recruit community controls at the residency of one *S. suis* case because of the distance from the study site (Central Viet Nam, 800 kms from Ho Chi Minh City). During the period of recruitment we therefore included 101 cases of confirmed *S. suis meningitis*, 303 hospital controls and 300 community controls for analysis (Figure 5-1).

Figure 5-1 Flow diagram of inclusion of study participants[1]



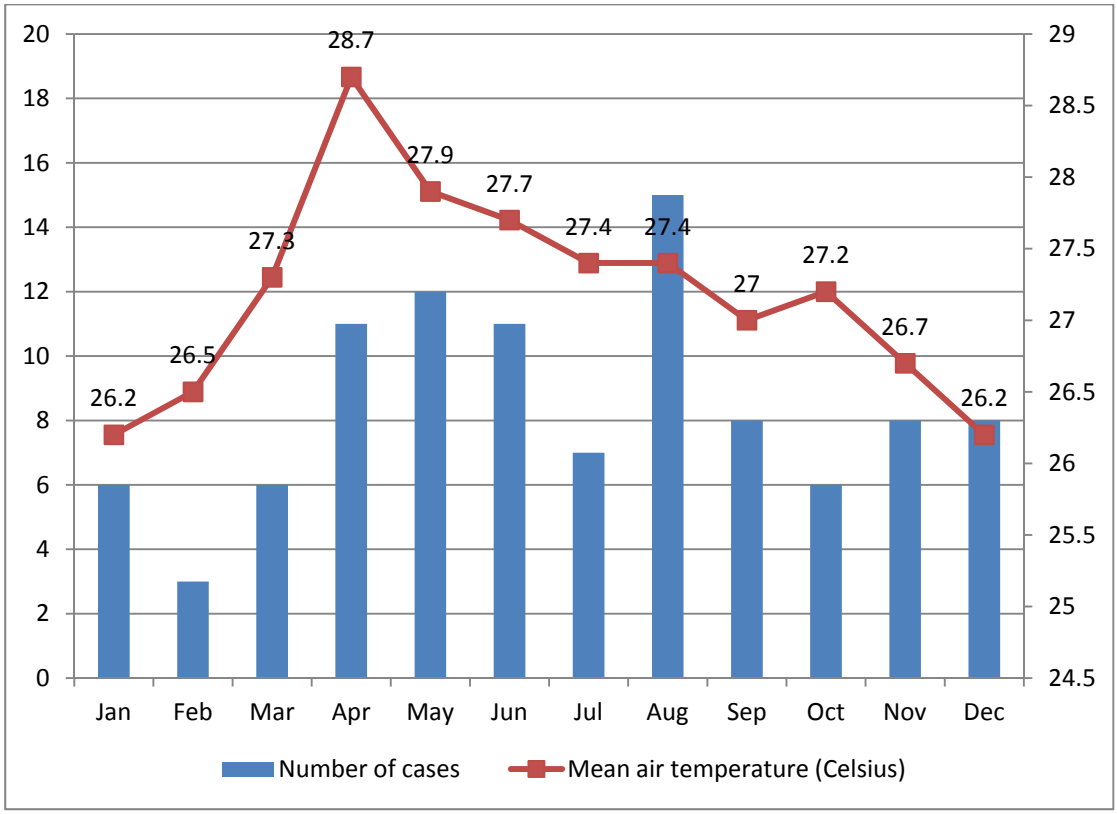
[1] **Reasons for exclusions of cases:** HIV (+) (3), transfer to other hospital because of presumed tuberculous meningitis (2), confusion more than 14 days after admission (1), language differences precluding interview (1).

Reasons for exclusion of controls: death (37), prolonged coma (72), unconfirmed bacterial meningitis (80), transfer to other hospitals (61), use of antimicrobial agents for more than 2 days in case of suspected viral encephalitis/meningitis (38), and absence of diagnosis of CNS infection (23). For one case, community controls could not be included because of too long distance of community to study site.

5.4.1 Characteristics of the participants

S. suis infection occurred sporadically throughout the year without any clear seasonality (Figure 5-2). Clustering of cases was not observed.

Figure 5-2 Distribution of *Streptococcus suis* meningitis cases and mean air temperature of southern Viet Nam in months during the study period (2006-2009)(GSO 2009)



Ninety-seven patients (96%) were infected with *S. suis* serotype 2 while only four patients (4%) were infected with other serotypes, including serotype 14 (3 cases) and untypeable serotype (1 case). *S. suis* cases were predominantly male (82%) and from a rural residence (81%) with a median (IQR) age of 50 (41-59) years. A high

proportion of cases had an occupation related to pigs (21%), other exposure to pigs (46%) or reported eating of "high risk" dishes in the last two weeks (48%). Except for fresh blood, other "high risk" dishes were mostly undercooked foods. Exposures to pigs/pork or eating "high risk" dishes were reported in 72/101 *S. suis* cases (71.29%), 91/303 hospital controls (30.03%) and 84/300 community controls (28%). Twenty-six *S. suis* cases (25.74%), compared to 52 hospital controls (17.16%) and 29 community controls (9.67%), only ate these "high risk" dishes without any other exposure to pigs or pork. Hospital and community controls were more frequently female and (unmatched) hospital controls were significantly younger, with a higher proportion of urban residence (Table 5-1). I further analyzed the age distribution of cases, hospital controls and non-*S. suis* bacterial meningitis patients in the hospital control group (Table 5-2). Nearly 70% of *S. suis* meningitis patients were older than 45 years old while only 25% of non-*S. suis* meningitis patients and 20% of hospital control group belonged to this age group. The 49 hospital controls with bacterial meningitis (not *S. suis*) were significantly younger than *S. suis* cases with median (IQR) age of 27 (23-45) compared to 50 (41-59) years ($p<0.001$).

Table 5-1 Characteristics of *Streptococcus suis* cases and controls

Characteristics	Cases (n=101)	Hospital controls (n=303)	Community controls (n=300)
Sex, n(%)			
- **Male**	83 (82.18)	202 (66.67)	169 (56.33)
- **Female**	18 (17.82)	101 (33.33)	131 (43.67)
Residence, n(%)			
- **Rural**	82 (81.19)	193 (63.70)	243 (81.00)
- **Urban**	19 (18.81)	110 (36.30)	57 (19.00)
Age (years), median (interquartile range)	50 (41,59)	27 (20,40)	50 (41,59)
Occupations related to pigs[(1)], n(%)	21 (20.79)	8 (2.64)	8 (2.67)
Education level, n(%)			
- **Primary**	52 (51.49)	96 (31.68)	175 (58.33)
- **Secondary "level 2"**	30 (29.70)	134 (44.22)	73 (24.33)
- **Secondary "level 3"**	11 (10.89)	40 (13.20)	30 (10)
- **University**	1 (0.99)	21 (6.93)	2 (0.67)
- **Illiterate**	6 (5.94)	12 (3.96)	19 (6.33)
Religion			
- **Buddhism**	64 (63.37)	160 (52.81)	181 (60.33)
- **Catholicism**	12 (11.88)	42 (13.86)	31 (10.33)
- **Protestant**	1 (0.99)	4 (1.32)	0
- **Cao Dai**	7 (6.93)	17 (5.61)	29 (9.67)
- **Other**	2 (1.98)	1 (0.33)	6 (2)
- **No**	15 (14.95)	77 (25.41)	53 (17.67)
Ethnic background			
- **Kinh**	99 (98.02)	287 (94.72)	298 (99.33)
- **Khmer**	1 (0.99)	4 (1.32)	2 (0.67)

- **Chinese**	1 (0.99)	4 (1.32)	0
- **Other**	0	8 (2.64)	0
Medical history, n(%)			
- **Diabetes mellitus**	3 (2.97)	3 (0.99)	4 (1.33)
- **Alcoholism**	14 (13.86)	18 (5.94)	20 (6.67)
- **Splenectomy**	1 (0.99)	0	0
Skin injuries, n(%)	33 (32.67)	18 (5.94)	11 (3.67)
Breeding pigs at home, n(%)	23 (22.77)	33 (10.89)	41 (13.67)
Any exposure to pigs/pork in the last 2 weeks, n(%)	46 (45.54)	39 (12.87)	55 (18.33)
- **With skin injuries**	20 (19.80)	5 (1.65)	2 (0.67)
- **Without skin injuries**	26 (25.74)	34 (11.22)	53 (17.67)
Eating any "high risk" dish in the last 2 weeks [(2)]	48 (47.52)	66 (21.78)	48 (16.00)
- Fresh pig blood	5 (4.95)	5 (1.65)	5 (1.67)
- Tonsils/tongue	19 (18.81)	29 (9.57)	25 (8.33)
- Stomach/intestines	45 (44.55)	53 (17.49)	42 (14)
- Uterus	8 (7.92)	8 (2.64)	13 (4.33)
- Undercooked pig blood	11 (10.89)	18 (5.94)	5 (1.67)
Only eating "high risk" dishes (without any other exposure to pigs/pork)	26 (25.74)	52 (17.16)	29 (9.67)
Ill pigs at home in the last 4 weeks, x/n (%)	10/23 (43.48)	1/33 (3.03)	0/40 (0)
Pigs at home carrying *S. suis* serotype 2 (confirmed by PCR), x/n [(3)] **(%)**	9/22 (40.91)	3/13 (23.08)	5/28 (17.86)

[(1)] Butcher, pig breeder, slaughterer, roaster, meat transporter, meat processing, veterinarian and cook
[(2)] Fresh pig blood, tonsils, tongue, stomach, intestines, uterus, unwell-cooked blood
[(3)] n was the number of participant's houses where the pigs' samples were taken

Table 5-2 Age distribution of *Streptococcus suis* cases and hospital controls

Age groups	Cases	Hospital controls	BM [(1)] (not *S. suis*) in hospital controls
<30	5 (4.95)	173 (57.10)	27 (55.10)
30 – 44	29 (28.71)	78 (25.74)	9 (18.37)
45 – 59	45 (44.55)	33 (10.89)	8 (16.33)
60 – 74	15 (14.85)	16 (5.28)	3 (6.12)
75+	7 (6.93)	3 (0.99)	2 (4.08)

[(1)] Bacterial meningitis

5.4.2 Analysis of risk factors

Occupations related to pigs, breeding pigs at home, exposures to pigs or pork with skin injuries, eating "high risk" dishes in the last 2 weeks and having ill pigs at home in the last 4 weeks were associated with *S. suis* meningitis, after adjustment for potential confounders (residency, age and sex) (Table 5-3 and 5-4). Alcoholism was significantly associated with *S. suis* without adjustment (crude odd ratio [OR] in hospital control group, 2.55 and 95% confidence interval [CI], 1.22 to 5.33 and crude OR in community control group, 2.50 and 95%CI, 1.15 to 5.45) but the association disappeared after adjustment.

Table 5-3 Risk factors of *Streptococcus suis* infection on univariate analysis (Cases versus Hospital controls)

Exposures	Cases versus Hospital controls			
	OR [1] (95%CI)	p value	OR[2] (95%CI)	p value
Occupations related to pigs	9.68 (4.13-22.67)	<0.001	7.51 (2.85-19.82)	<0.001
Medical history				
- **Diabetes mellitus**	3.06 (0.61-15.41)	0.175	0.82 (0.13-5.23)	0.830
- **Alcoholism**	2.55 (1.22-5.33)	0.013	1.31 (0.54-3.16)	0.547
Skin injuries	7.68 (4.08-14.46)	<0.001	8.16 (3.72-17.92)	<0.001
Breeding pigs at home	2.41 (1.34-4.35)	0.003	2.34 (1.09-5.00)	0.028
Any exposure to pigs/pork in the last 2 weeks	5.66 (3.38-9.49)	<0.001	4.69 (2.43-9.07)	<0.001
- **With skin injuries**	14.72 (5.36-40.42)	<0.001	12.16 (3.74-39.50)	<0.001
- **Without skin injuries**	2.74 (1.55-4.86)	0.001	2.06 (0.99-4.27)	0.052
Eating any "high risk" dish in the last 2 weeks	3.25 (2.02-5.24)	<0.001	2.48 (1.35-4.52)	0.003
Ill pigs at home in the last 4 weeks [3]	24.62 (2.85-212.24)	0.004	30.10 (2.72-333.64)	0.006
Pigs at home with *S. suis* serotype 2 (confirmed by PCR)[4]	2.31 (0.49-10.82)	0.289	7.83 (0.68-90.19)	0.099

[1] Crude OR based on logistic regression.
[2] Adjusted for age, sex and rural/urban residence, using logistic regression.
[3] Only individuals with pigs at home were analyzed.
[4] Only individuals who had pig swab samples taken at their houses were analyzed.

Table 5-4 Risk factors of *Streptococcus suis* infection on univariate analysis (Cases versus Community controls)

Exposures	Cases versus Community controls			
	OR [1] (95%CI)	p value	OR[2] (95%CI)	p value
Occupations related to pigs	11.50 (4.31-30.65)	<0.001	11.01 (4.03-30.12)	<0.001
Medical history				
- **Diabetes mellitus**	2.25 (0.50-10.05)	0.288	3.75 (0.75-18.73)	0.107
- **Alcoholism**	2.50 (1.15-5.45)	0.021	1.48 (0.63-3.31)	0.381
Skin injuries	22.09 (7.79-62.64)	<0.001	22.30 (7.55-65.84)	<0.001
Breeding pigs at home	1.95 (1.04-3.65)	0.036	1.99 (1.04-3.80)	0.036
Any exposure to pigs/pork in the last 2 weeks	4.51 (2.55-7.97)	<0.001	4.16 (2.30-7.52)	<0.001
- **With skin injuries**	30 (7.01-128.35)	<0.001	26.95 (6.14-118.23)	<0.001
- **Without skin injuries**	1.66 (0.92-3.00)	0.090	1.57 (0.85-2.91)	0.152
Eating any "high risk" dish in the last 2 weeks	6.00 (3.33-10.81)	<0.001	4.38 (2.72-8.08)	<0.001
Ill pigs at home in the last 4 weeks [3]	-	-	-	-
Pigs at home with *S. suis* serotype 2 (confirmed by PCR)[4]	-	-	-	-

[1] Crude OR conditional logistic regression.
[2] Adjusted for sex (matched for age and residence), using conditional logistic regression.
[3] Only individuals with pigs at home were analyzed. OR could not be analyzed for community controls because none of them reported ill pigs at home
[4] Only individuals who had pig swab samples taken at their houses were analyzed. OR could not be analyzed for community controls because there was no discordant pair included in the analysis.

S. suis infection was independently associated with occupations related to pigs, exposures to pigs or pork in the presence of skin injuries in the 2 weeks prior to infection, and eating "high risk" dishes in the 2 weeks prior to infection after multivariate analysis. These associations were found in comparisons of cases with the hospital control group as well as with the community control group. Breeding pigs at home, diabetes mellitus, alcoholism or exposure to pigs or pork without skin injuries were not associated with *S. suis* infection in multivariate analysis (Table 5-5). In a sensitivity analysis, which included only male cases and controls, exactly the same risk factors were significant with similar odds ratios as in the main analysis. Risk factors identified by multivariate analysis of male cases and controls from either control group included eating "high risk" dishes (OR_1=3.46; 95%CI=[1.65-7.28] and OR_2=4.79; 95%CI=[2.02-11.40]), occupations related to pigs (OR_1=6.33; 95%CI=[1.55-25.79] and OR_2=7.46; 95%CI=[1.56-35.74]), and exposures to pigs or pork in the presence of skin injuries (OR_1=5.81; 95%CI=[1.07-31.48] and OR_2=7.11; 95%CI=[1.00-50.54]).

Table 5-5 Risk factors of *Streptococcus suis* infection - multivariate analysis

Exposure	Cases versus Hospital controls		Cases versus Community controls	
	OR_1 (95%CI)	**p value**	**OR_2 (95%CI)**	**p value**
Occupations related to pigs	3.84 (1.32-11.11)	0.013	5.52 (1.49-20.39)	0.010
Medical history				
- **Diabetes mellitus**	1.10 (0.17-7.31)	0.918	4.11 (0.78-21.68)	0.095
- **Alcoholism**	1.02 (0.38-2.73)	0.969	0.72 (0.24-2.14)	0.553
Breeding pigs at home	1.02 (0.39-2.69)	0.965	0.83 (0.34-2.03)	0.681
Any exposure to pigs/pork in the last 2 weeks				
- **With skin injuries**	7.48 (1.97-28.44)	0.003	15.96 (2.97-85.72)	0.001
- **Without skin injuries**	2.15 (0.88-5.24)	0.092	1.14 (0.49-2.69)	0.757
Eating any "high risk" dish in the last 2 weeks	2.22 (1.15-4.28)	0.017	4.44 (2.15-9.15)	<0.001
Rural	2.39 (1.13-5.04)	0.022	-	-
Age (by +10 years)	2.59 (2.04-3.29)	<0.001	-	-
Male sex	4.47 (1.88-10.64)	0.001	3.53 (1.59-7.82)	0.002

OR_1: Odd ratio between cases and hospital controls
OR_2: Odd ratio between cases and community controls

5.4.3 Carriage of *S. suis* serotype 2 in pigs

We collected 571 pig swab samples from pigs present around the house of 22 of 23 cases, 28 of 41 community controls and 13 out of 33 hospital controls respectively, who kept pigs around the house (Table 5-1). Median of herd size was 7 pigs (range, 1 to 50) and *S. suis* serotype 2 was detected in 9 (41%) case group herds, 3 (23%) hospital control group herds, and 5 (18%) matched community control group herds. Differences between case group and hospital control group were not statistically significant. The odd ratio could not be analyzed for community controls because there was no discordant pair included in the analysis. (Table 5-3 and 5-4).

5.5 Discussion

We conducted the largest prospective epidemiological assessment of risk factors of *S. suis* infection globally. In addition to the previously suggested risk factors, such as occupational exposure and contact with pigs or pork without skin protection, we identified the ingestion of food with a high risk of contamination with *S. suis* serotype 2 to be an important risk factor for *S. suis* serotype 2 meningitis. To investigate eating habits as a risk factor of *S. suis* infection, we focused on potential "high risk" dishes common in Viet Nam. These include fresh or under-cooked blood, tonsils, tongue, stomach, intestines and uterus. Such food items typically are undercooked when eaten as a main dish (as opposed to as components of well cooked main dishes such as rice or noodle soups), as was generally the case in our patients. By multivariate analysis including all potential risk factors and confounding factors, we demonstrated that eating these "high risk" dishes in the 2 weeks prior to admission was a significant risk factor for this infection with OR_1, 2.22 (95%CI, 1.15-4.28) and

OR_2, 4.44 (95%CI, 2.15-9.15). Eating habits were also confirmed as a risk factor in a case-control study conducted in Phayao province, Thailand. In this outbreak, patients attended a funeral ceremony, where fresh pig blood and raw pork were served. In the multivariate analysis, only eating blood remained statistically significant with an adjusted OR of 24.8 (95%CI, 1.46-423.53). However, the sample size of this study was small with 9 infected individuals and 36 controls (Khadthasrima et al. 2009). A history of raw pork or uncooked pig blood consumption prior to illness was reported in 25/40 patients (62.5%), while a history of direct skin contact with pigs or raw pork products was only recorded in 10/40 patients (25%), in another study also conducted in northern Thailand (Navacharoen et al. 2009). Entry of *S. suis* through gastrointestinal tract might explain why diarrhoea was one of the presenting symptoms documented in 13/61 (21%) of patients from Hong Kong (Kay et al. 1995). However, eating pork was not associated with *S. suis* cases in a matched case-control study conducted during the outbreak in Sichuan province in 2005 (Yu et al. 2005). *S. suis* lives as normal flora in the upper respiratory, gastrointestinal and genital tract of pigs and can cause invasive disease in pigs. Pork and pig's organs can also be contaminated in slaughter houses. *S. suis* was isolated from 7 out of 117 (6.1%) of raw pork meat samples from 3 out of 6 wet markets in 6 districts in Hong Kong (Ip et al. 2007). There may be a high bacterial load in food items that are kept at high ambient temperatures in the tropics for example when being sold in the markets. Therefore, patients may be infected with *S. suis* through the gastrointestinal tract if the "high risk" dishes are served as raw or under-cooked food.

Reports from China, Thailand and the northern provinces of Viet Nam, suggested *S. suis* infections tend to present in the summer or the rainy season

potentially linked with a higher incidence of disease in pigs due to high temperature and humidity (Kay et al. 1995; Huang et al. 2005; Wangkaew et al. 2006; Yu et al. 2006; Navacharoen et al. 2009; Wertheim et al. 2009). However in our study seasonal variation was not observed. (Figure 5-6).

I found that age of *S. suis* meningitis patients was higher than that of patients with bacterial meningitis caused by other bacteria and that high age was associated with increased risk of infection with *S. suis*. In contrast, paediatric infections with *S. suis* are extremely rare, presumably related to a lack of exposure associated with increased risk of *S. suis* infection, in children. Breeding as well as slaughtering pigs are the hard works which are more suitable for males than females. Moreover, eating the "high risk" dishes, such as fresh blood and raw or undercooked pork, usually reported in adult males. These reasons could explain why high male-to-female ratio was documented in human *S. suis* infection.

The association between human *S. suis* infection and occupational exposures to pigs or pork has been reported since 1968. Most of these reports have been in pig breeders or abattoir workers (Arends et al. 1988; Walsh et al. 1992). In The Netherlands, the risk of *S. suis* meningitis among these individuals was estimated to be 3/100,000 people. This risk was 1500 times higher than that of persons not working in the pork industry (Arends et al. 1988). The annual incidence of *S. suis* infection among the general population in Hong Kong was calculated as 0.09/100,000 people while this incidence among occupational group was reported to be 32/100,000 (Ma et al. 2008). In Viet Nam, the proportion of patients reported to have occupational exposures was lower than reported in European patients but it remained an important independent risk factor with OR_1, 3.84 (95% CI, 1.32-11.11) and OR_2,

5.52 (95% CI, 1.49-20.39). Risk of infection may be different in different types of exposure. A matched case-control study was conducted in the Sichuan outbreak, including 29 cases and 147 controls. The authors found that slaughtering (OR, 11.978; 95%CI, 3.335-42.756) and carcass cutting and processing (OR, 3.008; 95%CI, 1.022-8.849) sick or dead pigs were associated with *S. suis* cases (Yu et al. 2005).

A number of reports have linked skin injuries as a factor in acquisition of infection in individuals in direct contact with pigs or pork. Significant skin injury, sometimes with signs and symptoms of bacterial infection, was evident in 5 out of 35 (14%) of people with *S. suis* in the UK, 4 out of 15 cases (16%) in Hong Kong and 104 out of 215 cases (48%) in Sichuan province's outbreak (Walsh et al. 1992; Kay et al. 1995; Yu et al. 2006). In this study, skin injuries were reported in 33/101 (33%) of *S. suis* patients compared to 18/303 (6%) of hospital controls and 11/300 (4%) of community controls (Table 5-5). These often relatively minor skin injuries may allow direct entry of the bacteria in people with direct contact with infected pigs or pork. Contact with pigs within the last two weeks and skin lesions was associated with a significant high risk of infection with OR_1, 7.48 (95%CI, 1.97-28.44) and OR_2, 15.96 (95%CI, 2.97-85.72) (Table 5-9). Skin injuries were most often recorded in slaughter house workers, cooks and housewives involved in processing meat. Skin protection, including gloves, hand washing and exclusion of people with obvious skin lesions from direct contact with pigs and pork meat may help to reduce the incidence of the disease.

Pig carriage of *S. suis* serotype 2 was higher in the case group than in hospital control and in community control groups but it was not statistically significant. This condition reflected the bacteria as a common commensal or pathogen for pigs.

Nevertheless, information on the prevalence of sick pigs at the participant's house may have been underestimated, especially in the community control group. Patients and their relatives may have been reluctant to admit to having sick pigs at home for fears of losing their pigs to the veterinary authorities or not being able to sell their stock at a good price. We were unable to take pig samples immediately after the patient was admitted to HTD, as there was an inevitable delay until we could travel to their home. The median time to conduct the home visit was 22 days (IQR, 18-32) after admission. It is possible that in this gap the pigs we surveyed in the home were not the same pigs that may have lead to the infection that caused the admission.

5.6 Conclusions

In conclusion, *S. suis* is an important and emerging public health issue in Asia and one with the potential for both endemic transmission and for explosive epidemics. I identified risk factors for *S. suis* infection which can be addressed in health education programs targeted at individuals and communities at risk, focusing on skin protection for those in direct contact with pigs or pork and avoiding eating raw or under-cooked pig products.

Chapter 6

Human carriage of *Streptococcus suis* serotype 2

6.1 Introduction

S. suis can be a commensal or pathogen for a wide range of mammalian species, such as cattle, sheep, goats, horse, cat, dog, and particularly pigs (Staats et al. 1997). Pigs harbour *S. suis* in their tonsils and multiple serotypes and untypeable strains may be present in a single herd and even within the same animal (Martinez et al. 2002; Marois et al. 2007). The bacteria have also been recovered from the nasal cavity, reproductive, and alimentary tract of subclinical carriers (Robertson et al. 1989; Brisebois et al. 1990; Gottschalk et al. 2000). Tonsillar swab cultures from live pigs indicated that carrier rates of *S. suis* serotype 2 in herds vary from 0 to 100% (Mwaniki et al. 1994). Carriers of *S. suis* serotype 2 are infectious to other pigs and are significant in transmission of pathogenic strains via close contact or exposure to the pathogen in aerosol form (Staats et al. 1997; Madsen et al. 2001; Huang et al. 2005). In humans, *S. suis* causes a systemic infection that affects several organ systems, such as bacterial meningitis, streptococcal toxic shock syndrome, arthritis and bacterial endocarditis (Lun et al. 2007; Wertheim et al. 2009). As mentioned before, this zoonosis is considered an occupational disease and the common occupations at risk are pig breeder, abattoir worker, those involved in meat processing and meat transport, butcher, and cooks (Arends et al. 1988; Walsh et al. 1992; Kay et al. 1995). It is hypothesized that patients may be infected through minor cuts or abrasions on their skin (Arends et al. 1988; Yu et al. 2006). However, although occupational exposure to pigs or pork was documented in 88% of the European

patients described, it was reported in only up to 42% of the Asian cases (Kay et al. 1995; Mai et al. 2008). Significant skin injury was only evident in 5 out of 35 (14%) of people with *S. suis* in the UK, 4 out of 15 cases (16%) in Hong Kong and 104 out of 215 cases (48%) in Sichuan province's outbreak (Walsh et al. 1992; Kay et al. 1995; Yu et al. 2006). Whilst it is known that pigs can carry *S. suis* asymptomatically, it is not known if there is asymptomatic carriage of *S. suis* in the respiratory or gastrointestinal tract of humans, which may lead to person-to-person transmission or potentially to infection. *S. suis* was cultured from pharyngeal swabs from 7 of 132 (5%) German workers in pig slaughterhouses and pork dissecting and processing industry but was not isolated from 130 controls from the same geographic area. Carriage was confirmed in all four cases where repeat samples were available (Strangmann et al. 2002). Hence, I conducted a study to identify human carriage of *Streptococcus suis* serotype 2 in the upper respiratory and gastrointestinal tract. As detection of potential carriage in cases and hospital controls could be affected by their antimicrobial treatment, household members were also studied for carriage of *S. suis* serotype 2 since if carriage and associated transmission were to occur, household members of carriers are the most likely to become positive.

6.2 Aims

1. To describe pig exposures and human carriage of *S. suis* serotype 2 in patients with meningitis caused by *S. suis* serotype 2 and controls.
2. To describe pig exposure and human carriage of *S. suis* serotype 2 in household members of cases and controls.

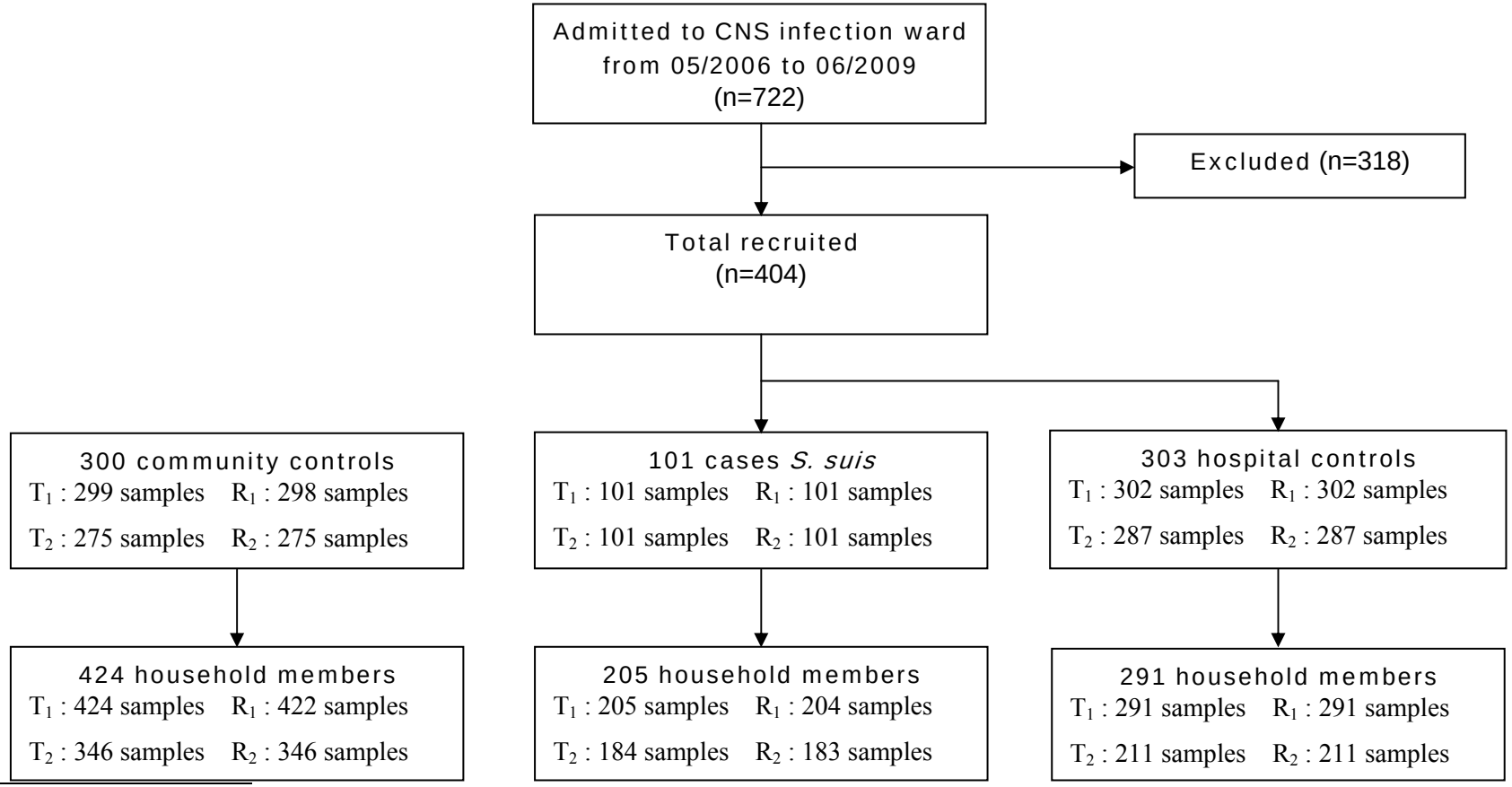
6.3 Materials and Methods

Materials and Methods are presented in Section 2.3.2, 2.4.3.1, 2.4.5 and 2.5.

6.4 Results

A total of 722 patients with suspected CNS infection or severe malaria, were admitted to HTD between May 2006 and June 2009. Three hundred and eighteen patients were excluded because they did not fulfill the inclusion criteria for the case-control study, such as death within 14 days, prolonged coma, unconfirmed bacterial meningitis, transfer to other hospitals and use of antimicrobial agents more than 2 days in case of suspected viral encephalitis/meningitis (Table 5-1 and Table 5-2). During this period, 101 patients with confirmed *S. suis* meningitis were recruited, 303 hospital controls and 300 community controls. Two hundred and five household members of *S. suis* cases, and 291 and 424 hospital controls and community controls, respectively were recruited. It was not possible to take throat and rectal swab samples of one patient in the hospital control group and another person in the community control group because they refused sampling. The number of second samples was lower than of the first samples because patients' hospitalized duration was less than 10 days or the community controls and household members were absent at the second visit (Figure 6-1).

Figure 6-1: Flow diagram of inclusion of study participants[2]



[2] T_1, R_1: First throat (T) and rectal (R) swab sample, taken on hospital admission or on the first household visit
T_2, R_2: Second throat (T) and rectal (R) swab sample, taken after admission 10-14 days or on the second household visit (12-16 days after the first visit)

6.4.1 Pig exposure and carriage results of case and control groups

Pigs or pork exposure was reported more frequently in the case group than in the hospital and community control groups. Nearly 20% of cases had occupations related to pigs or pork, but only 2-3% of hospital or community controls had such occupations. Similarly, contacts with pigs or pork in the last 2 weeks were recorded in 45% of cases, compared to 13% and 18% of hospital controls and community controls, respectively. Eating the "high risk" dishes in previous 2 weeks, such as fresh blood, undercooked pig's intestines and stomach, was also documented in nearly 50% of case group and 15-20% of control groups. However, the proportion of cases and controls in the two control groups who reported breeding pigs at home was not significantly different, 20% compared to 11-14% (Table 6-1).

Table 6-1 Exposure to pigs or pig products of cases and controls and PCR results of their throat and rectal swab samples

Characteristics	Cases (n=101)	Hospital controls (n=303)	Community controls (n=300)
Occupations, n (%)			
- **Butcher**	5 (4.95)	2 (0.66)	0 (0)
- **Pig breeder**	6 (5.94)	2 (0.66)	6 (2)
- **Slaughterer/pig's roaster**	7 (6.93)	0 (0)	1 (0.33)
- **Meat transporter**	1 (0.99)	0 (0)	0 (0)
- **Meat processing**	1 (0.99)	0 (0)	0 (0)

- **Veterinarian**	1 (0.99)	0 (0)	0 (0)
- **Cook**	0 (0)	4 (1.32)	1 (0.33)
- **No occupational exposure to pigs**	80 (79.21)	295 (97.36)	292 (97.33)
Pigs at home, n (%)	23 (22.77)	33 (10.89)	41 (13.67)
Contact with pigs/pork in the last 2 weeks, n (%)	46 (45.54)	39 (12.87)	55 (18.33)
- **Bathing pigs**	18 (17.82)	17 (5.61)	30 (10)
- **Feeding**	19 (18.81)	18 (5.94)	29 (9.67)
- **Cleaning up piggery**	19 (18.81)	13 (4.29)	25 (8.33)
- **Slaughtering**	13 (12.87)	0 (0)	1 (0.33)
- **Contact blood/pig's organs**	30 (29.70)	7 (2.31)	18 (6)
- **Visiting pig's farm**	17 (16.83)	17 (5.61)	16 (5.33)
Eating "high risk" dishes in the last 2 weeks, n (%)	48 (47.56)	66 (21.78)	48 (16)
- **Fresh blood**	5 (4.95)	5 (1.65)	5 (1.67)
- **Pig tonsils/tongue**	19 (18.81)	29 (9.57)	25 (8.33)
- **Pig intestines/stomach**	45 (44.55)	53 (17.49)	42 (14)
- **Pig uterus**	8 (7.92)	8 (2.64)	13 (4.33)
- **Undercooked blood**	11 (10.89)	18 (5.94)	5 (1.67)
Positive throat/rectal swab samples by PCR, x/n (%)			

- **1st throat swab**	1/101 (0.99)	0/302 (0)	0/299 (0)
- **1st rectal swab**	5/101 (4.95)	0/302 (0)	0/298 (0)
- **2nd throat swab**	0/101 (0)	0/287 (0)	0/275 (0)
- **2nd rectal swab**	1/101 (0.99)	0/287 (0)	0/275 (0)

S. suis serotype 2 was detected in the throat or rectal swab samples of six *S. suis* serotype 2 meningitis patients by PCR. One patient had a positive throat swab sample on admission day and one other patient had two positive rectal swab samples, one on admission day and one 8 days later. The remaining patients only had positive rectal swab samples on admission day (Table 6-1). *S. suis* serotype 2 was cultured from the throat swab sample and one of the rectal swab samples. All of the patients with PCR positive swab samples were male, middle-aged persons and lived in the Mekong river delta of southern Viet Nam. None of them had a history of splenectomy or diabetes mellitus but for two patients alcoholism was reported. Three of these six patients had pigs at home. No illness was reported in these pigs in the last 4 weeks prior to presentation of the patients and the PCR results of pig tonsil swab samples were negative indicating that these pigs did not carry *S. suis* serotype 2 in their tonsils. None of these patients had skin injuries. Three patients had eaten pig intestines within the two weeks prior to admission. Of these three patients, the throat swab sample was positive in one patient and rectal swab samples were positive in the two others. One of these two patients had two PCR positive rectal swab samples on separate occasions. *S. suis* serotype 2 was cultured from the first sample (Table 6-2).

It was not possible to detect DNA of *S. suis* serotype 2 in the throat and rectal swab samples of 601 hospital and community controls.

Table 6-2 Epidemiological characteristics of six patients with *S. suis* serotype 2 detected in throat swab or rectal swab samples by PCR

Characteristics	Patients with PCR positive swab samples (n=6)
Male sex, n (%)	6 (100)
Age (years), median (range)	54.5 (40,79)
Rural residency	5 (83.33)
History of splenectomy	0 (0)
Alcoholism	2 (33.33)
Diabetes mellitus	0 (0)
Occupation related to pigs [1]	1 (16.67)
Breeding pigs at home	3 (50)
Contact with pigs/pork in the previous 2 weeks [2]	2 (33.33)
Eating "high risk" dishes in the previous 2 weeks [3]	3 (50)
Contact with pigs/pork or eating "high risk" dishes in the previous 2 weeks	5 (83.33)
Skin injuries	0 (0)

[1] Butcher, slaughterer, pig's breeder, meat transporter, vet, cook and meat process

[2] Bathing, feeding, cleaning up piggery, slaughtering, direct contact blood/pig's organs and visiting pig's farm

[3] Fresh blood, undercooked tonsils/tongue, intestines, stomach and uterus

Patients with PCR positive swab samples had a short history of illness with a median of 4 days. Five of six patients had altered level of consciousness but none of them got seizures. Gastrointestinal symptoms were common, including vomiting in 5 of 6 patients and diarrhoea in 2 of 6 patients but none had gastrointestinal bleeding. Four of six patients had low blood platelet count (<100,000/μl) and two patients suffered from skin haemorrhages. One or two doses of intravenous ceftriaxone or cefotaxime were given to 3 patients before admission and antibiotic treatment was not recorded in 2 other patients. However, *S. suis* serotype 2 was cultured from blood in 4 patients and from CSF in 5 patients (Table 6.3).

Table 6-3 Clinical manifestations of six patients with *S. suis* serotype 2 detected in throat swab or rectal swab samples by PCR

Characteristics	Patients with PCR positive swab samples (n=6)
Days of illness (before admission), median (range)	4 (2,8)
Confusion	5 (88.33)
Glasgow Coma Scale (GCS), median (range)	12 (9,15)
Seizures	0 (0)
Vomiting	5 (88.33)
Diarrhoea	2 (33.33)
Gastrointestinal haemorrhage	0 (0)
Skin haemorrhages	2 (33.33)
Platelet count ($x10^3/\mu l$), median (range)	51.65 (20.3, 210)
No antibiotic therapy before admission [1]	1 (16.67)
***S. suis* serotype 2 cultured from blood**	4 (66.67)
***S. suis* serotype 2 cultured from cerebrospinal fluid (CSF)**	5 (83.33)
***S.suis* serotype 2 detected in CSF by real-time PCR** [2]	5 (83.33)

[1] Antibiotic therapy before admission was not reported clearly in 2 patients and these patients were assigned as "unknown".

[2] Real-time PCR was not done in one patient because his CSF sample was not sent for PCR but *S. suis* serotype 2 was cultured from CSF and blood of this patient.

6.4.2 Pig exposure and carriage results of household members of case and control groups

A total of 3318 throat and rectal swab samples were collected from 205, 291 and 424 household members of cases, hospital controls and community controls, respectively. Nearly fifty percent of these household members were farmers or housewives. Occupations related to pigs or pork in this group included predominantly butcher, pig breeder or cook but relative frequencies were below 5%. Some of the household members had direct contact with pigs or pork and all of the household members of *S. suis* patients had direct contact with patients, such as living in the same house or taking care of the patient. However, DNA of *S. suis* serotype 2 was not detected in any of the swab samples of these household members (Table 6-4).

Table 6-4 Characteristics of household members and PCR results of their throat swab and rectal swab samples

Characteristics	House hold members		
	Case group (n=205)	**Hospital control group (n=291)**	**Community control group (n=424)**
Age (years), median (interquartile range)	36 (24,50)	41 (29,49)	39 (25,51)
Occupations, n (%)			
- **Butcher**	10 (4.88)	1 (0.34)	2 (0.47)
- **Pig breeder**	5 (2.44)	2 (0.69)	5 (1.18)
- **Slaughterer**	3 (1.46)	0 (0)	0(0)
- **Cook (related to pork)**	7 (3.41)	3 (1.03)	11 (2.59)
- **Housewife**	26 (12.68)	54 (18.26)	78 (18.40)
- **Farmer**	53 (25.85)	112 (38.69)	151 (35.61)
- **Others (unrelated to pigs/pork)**	100 (48.78)	111 (38.24)	175 (41.27)
- **Unknown job**	1 (0.49)	8 (2.75)	2 (0.47)
Pigs at home, n (%)	54 (26.34)	44 (15.12)	59 (13.92)
PCR positive throat/rectal swab samples, x/n (%)			
- **1st throat swab**	0/205 (0)	0/291 (0)	0/424 (0)
- **1st rectal swab**	0/204 (0)	0/291 (0)	0/422 (0)
- **2ndthroat swab**	0/184 (0)	0/211 (0)	0/346 (0)
- **2nd rectal swab**	0/183 (0)	0/211 (0)	0/346 (0)

6.5 Discussion

Though asymptomatic carriage is common in healthy pigs, it is unknown whether there is asymptomatic human carriage of *S. suis*. All common human pathogens causing bacterial meningitis are typically carried, either in the upper respiratory tract (*S. pneumoniae, H. influenza* type b and *N. meningitides*) or in the intestine or vagina (*E. coli, Listeria spp* and *Streptococcus* group B). All of these bacteria are encapsulated microorganism like *S. suis*. If human carriage of *S. suis* occurred, this would be very important to know as it may play a role in the routes of transmission, the risk of infection and evolution of the bacteria.

In this study, it was not possible to demonstrate *S. suis* serotype 2 carriage in 1521 healthy persons or patients without *S. suis* infection, including those with pig exposures such as abattoir worker, butcher and pig breeder. This finding indicates that humans are either not asymptomatic carriers of *S. suis* serotype 2 or it is extremely uncommon. In contrast, the nasopharyngeal carriage rate of *S. suis* in a high risk group, consisting of butchers, abattoir workers and meat processors, was 7 out of 132 (5.3%), while none of those without contact with pigs or pork were found to be carriers, in one study in Germany (Strangmann et al. 2002). However, the serotype of these *S. suis* strains was not reported in this study. In this study I only tested for the presence of serotype 2 strains as this is by far the predominant serotype causing disease in humans. There are other serotypes in pigs that could potentially be transmitted to and be carried by humans who are continuously exposed to pigs or their products, and carriage of such strains in the German population at high risk may explain differences in carriage rates observed.

In addition, enzyme linked immunosorbent assay (ELISA) was used to investigate the prevalence of antibodies to *S. suis* serotype 2 in sera of different occupational groups in New Zealand. In 9 of 96 (9.3%) dairy farmers, 11 of 107 (10.3%) meat inspectors, 15 of 70 (21.4%) pig farmers, and none of 16 veterinary students, antibodies against *S. suis* serotype 2 were detected (Robertson et al. 1989). In a study in the US, antibodies against *S. suis* serotype 2 were found in 7 out of 73 (9.6%) swine workers compared with 1 out of 67 (1.5%) of non-swine exposed participants. All of the positive cases had worked and lived in swine farms for over 10 years (Smith et al. 2008). Subclinical infection, rather than human carriage, with *S. suis* serotype 2 may occur in those with direct contact with pigs or pork, as suggested by these serological test results. However, these data should be regarded with caution because no standardized serological test to detect *S. suis* antibodies in humans or pigs exists, and the ELISA test used may detect cross reactivity with other antigens (Gottschalk et al. 2007). Further evidence for the absence of human carriage of *S. suis* serotype 2 in their respiratory and alimentary tract is the antimicrobial susceptibility of *S. suis* serotype 2 strains isolated from human. All of *S. suis* strains isolated from patients in Viet Nam, where antibiotic drugs can be bought without prescription, were totally susceptible to penicillin and ceftriaxone (Mai et al. 2008; Wertheim et al. 2009; Hoa et al. 2011).

Eating pig intestines in the few days prior to admission was reported in 3 of 6 patients with a PCR positive throat or rectal swab, two of which were also culture positive. Only one patient had a PCR positive throat swab sample and the bacteria were also isolated from this sample. He did not have any exposure to pigs or pork in the last 2 weeks, except eating undercooked pig's intestines in a wedding party two

days before admission. Taken together, rather than indicating human carriage of *S. suis* serotype 2, our results strengthen the hypothesis that the gastrointestinal tract may be a route of entry for *S. suis* serotype 2, for at least a proportion of patients.

6.6 Conclusions

After carefully investigating 1622 healthy persons or patients with or without *S. suis* serotype 2 infection, it was not possible to demonstrate asymptomatic carriage of *S. suis* serotype 2 in the respiratory or gastrointestinal tract of humans, with the exception of one patient in whom carriage cannot be excluded since consecutive rectal swab samples were PCR positive. These findings may reinforce the evidence for the alimentary tract as a route of entry of *S. suis* serotype 2 in the Asian population.

Chapter 7

Discussion

Central nervous system infection is a serious and life-threatening disease with high morbidity and mortality in developing countries. An increasing number of reports related to *Streptococcus suis* meningitis have appeared in the recent years, especially after the explosive outbreak of this infection in Sichuan province of China, raising the alarm about an emerging zoonotic disease in Southeast Asia (Gottschalk et al. 2010). Two case series from the Hospital for Tropical Diseases (HTD) in Ho Chi Minh City and the National Hospital for Tropical Diseases (NHTD) in Ha Noi showed that *S. suis* is the most important cause of purulent bacterial meningitis in adults in these tertiary referral hospitals (Mai et al. 2008; Wertheim et al. 2009). However, we did not have any information on the incidence and characteristics of *S. suis* meningitis in other, provincial, hospitals in Viet Nam. In addition, we were uninformed about the important risk factors of this infection in the Vietnamese population, with the exception of occupational exposure to pigs or pork, which was documented in 30% of patients in prior studies (Mai et al. 2008).

This thesis focused on the epidemiology of *S. suis* serotype 2 infection in Viet Nam and aimed to address the following questions:

1. Is human *S. suis* serotype 2 infection endemic across Viet Nam?
2. What are the epidemiological characteristics of human *S. suis* serotype 2 infection in Viet Nam, including incidence rate of disease, seasonality, and antimicrobial susceptibility of bacterial strains isolated from humans?

3. What are the main risk factors of human *S. suis* serotype 2 infection in Viet Nam?
4. Is there any human carriage of *S. suis* serotype 2 in the respiratory and alimentary tracts?

The extent to which these questions have been answered will now be discussed.

7.1 Human endemic disease in Viet Nam

S. suis infection in humans is sporadically reported in Western countries and may be underreported in China and Southeast Asian countries. Apart from the outbreaks in Jiangsu and Sichuan provinces in 1998 and 2005, any information about this infection in China is lacking. With the exception of reports from Thailand and Viet Nam, *S. suis* infection cases in other Southeast Asia countries, including Laos, Cambodia, Myanmar, Philippine, Brunei, Indonesia, Malaysia and Singapore, are rarely published in English literature. The reasons for this may be the really low incidence rate in the Muslim countries (Brunei, Indonesia and Malaysia) or the limitation of microbiological laboratories in isolating and identifying bacterial strains from cerebrospinal fluid and blood. In my opinion, the latter reason is probably the most important. Prior to this prospective surveillance study (01SS study) *S. suis* meningitis had never been reported from 2 central hospitals (Hue and Can Tho), 10 provincial hospitals (Ca Mau, Bac Lieu, Soc Trang, Tra Vinh, Dong Thap, An Giang, Kien Giang, Binh Phuoc, Dak Lak, Khanh Hoa) and 1 district hospital (Sa Dec). Following this study, *S. suis* meningitis cases has now been documented at all of the surveyed sites by using standard culture and real-time PCR methods. This pathogen was the most common cause of adult CNS infection as well as adult bacterial

meningitis (BM) in these hospitals, which accounted for 25% and 50% of cases, respectively. Similarly, *S. suis* infection was also confirmed in 151 of 450 (34%) of BM patients in another previous case series at HTD from 1996 to 2005, and in 108 of 268 (41%) of BM patients in our case-control study (EN study) at HTD from 2006-2009 (chapter 5), and in 50 of 57 (88%) of culture or PCR confirmed BM patients at NHTD in 2007 (Mai et al. 2008; Wertheim et al. 2009). Moreover, *S. suis* meningitis consistently accounted for at least 40% of bacterial meningitis cases at HTD in the period of 2000 – 2005 (Mai et al. 2008). Given the above evidence, it can be concluded that human *S. suis* infection is really an endemic disease in Viet Nam, with an incidence rate of 0.57 per 100,000 adult person-years. The incidence rate in the general Vietnamese population was 5 times higher than that of Hong Kong and 200 times higher than that of The Netherlands. *S. suis* infection is a public health problem which requires interventions in Viet Nam.

7.2 Epidemiological characteristics of human *S. suis* infection in Viet Nam

The majority of human *S. suis* cases reported in European countries sporadically occurred without any obvious seasonal pattern (Arends et al. 1988; Walsh et al. 1992). Other reports from the south of Viet Nam, including our case-control study, documented similar observations (Figure 5-2) (Mai et al. 2008). However, human infection cases tended to occur mainly during the summer or the rainy season in most of case series from China, Hong Kong, northern Thailand and northern Viet Nam (Kay et al. 1995; Wangkaew et al. 2006; Yu et al. 2006; Ma et al. 2008; Wertheim et al. 2009). In our prospective surveillance study, we found that the incidence of *S. suis* cases had a seasonal pattern in Hue Central hospital, but not in the

other hospitals which were located in the south of Viet Nam (Figure 4-1 and 4-2). As is shown in Figure 4-2, *S. suis* cases tended to occur when the mean air temperature was higher than 27^0C in Hue Central hospital. The lack of an obvious seasonal pattern of infection in the south of Viet Nam, (Figure 5-2), may be due to the fact that mean air temperatures are rarely below 26^0C in this geographical area with a tropical climate. *S. suis* infections tend to present in the summer or the rainy season, potentially linked with a higher incidence of disease in pigs due to high temperature and humidity (Kay et al. 1995; Huang et al. 2005; Wangkaew et al. 2006; Yu et al. 2006; Navacharoen et al. 2009; Wertheim et al. 2009). *S. suis* can survive better in the environment at higher temperatures and humidity (Staats et al. 1997). Pork may be contaminated after environmental contamination of *S. suis* in slaughterhouses or markets. *S. suis* was isolated from raw pork samples obtained from wet markets in Hong Kong (Ip et al. 2007). Potentially more important, pork which is colonized by S. suis as a result of pig carriage state, may acquire high bacterial load in pork sold at the markets in the condition of high temperature and humidity. In addition, sudden weather change, such as can be observed in the north of Viet Nam, can precipitate *S. suis* infection in pigs (Staats et al. 1997), potentially resulting in an increased sale of infected pork as farmers seek to sell pigs and pork before they loose their livelihoods.

The association between human *S. suis* infection and occupational exposures to pigs or pork was first suggested in 1968 (Arends et al. 1988; Walsh et al. 1992). However, exposure to pigs or pork was present in only 36/149 (24%) of patients in the 01SS study and in 46/101 (45%) of *S. suis* patients in the EN study, where the patients were carefully interviewed by two dedicated research nurses. Occupational exposure to pigs or pork was also reported in less than 50% of patients in another Asian case

series while the rate was nearly 90% in European patients (Kay et al. 1995; Mai et al. 2008). I hypothesized that consuming uncooked or partially cooked pork products maybe a risk factor for *S. suis* infection. Local culinary delights such as undercooked pig tonsils, intestines, uterus, and fresh pig blood, may also represent important sources of infection. In the case-control study, I provided evidence that local culinary habits were indeed a risk factor of *S. suis* infection in Viet Nam. In addition, *S. suis* serotype 2 DNA was detected in the rectal swab samples of five *S. suis* meningitis patients and three of these patients had eaten pig intestines in the two week prior to sampling. In addition, *S. suis* was cultured from two of these patients. Taken together, our results strengthen the hypothesis that the gastrointestinal tract may be a route of entry of *S. suis* serotype 2 for at least a proportion of patients. Whilst I am aware that epidemiological evidence does not directly prove a causal relationship, I consider this novel finding one of the most striking results of my thesis. These results are also supported by an outbreak investigation in Thailand, where *S. suis* infection in attendants of a funeral banquet was significantly associated with the consumption of raw pig blood. Clearly, more research is needed to establish this causal relationship.

In this thesis, a real-time PCR test was used to detect *S. suis* serotype 2 DNA in the CSF samples as well as in swab samples, which was more sensitive than culture method and had a detection limit of 1-5 colony forming units per reaction in a previous validation (Nga et al. 2011). Approximately, 30% (44/149) of *S. suis* cases in the 01SS study were only confirmed by PCR test, compared to 36/151 (24%) in a previous study at HTD and 11/41 (26%) in another study performed at NHTD in Ha Noi (Mai et al. 2008; Wertheim et al. 2009). Real-time PCR method is an effective procedure in surveillance of *S. suis* serotype 2 meningitis in developing countries,

where bacteriological culture facilities are limited and the use of antibiotics prior to admission is common. However, the cost of this method can be an obstacle for applying in the poor countries.

It has been reported that *S. suis* infection is extremely rare in children because of the absence of occupational exposure to pigs or pork (Gottschalk et al. 2007). In addition, children rarely eat fresh blood and raw or undercooked pork, which can be a potential source of infection. Despite using direct culture and sensitive PCR, it was not possible to detect any paediatric case of *S. suis* infection from 871 CSF samples.

Although asymptomatic carriage of *S. suis* is common in healthy pigs, it is unknown whether humans asymptomatically carry the bacteria. Human carriage could potentially contribute to an increased risk of infection, and to the possibility of person-to-person transmission. It was not possible to detect any *S. suis* serotype 2 DNA from the throat and rectal swab samples of 1521 healthy persons and non-*S. suis* infected patients, including those with pig exposures such as abattoir worker, butcher and pig breeders. This finding indicates that human carriage of *S. suis* serotype 2 must be extremely uncommon in this study population. I cannot exclude the possibility that other serotypes might be carried as the detection was limited to serotype 2. However, since 95% of culture confirmed *S. suis* infection is caused by serotype 2, such carriage is unlikely to contribute to any important morbidity.

7.3 Conclusions and future directions

I have the following conclusions on the epidemiology of *S. suis* serotype 2 infection in Viet Nam:

1. *S. suis* serotype 2 infection is an endemic disease in Viet Nam, which is responsible for 49% of cases of adult purulent bacterial meningitis with an average incidence rate of 0.57 per 100,000 adult person-years (95% CI, 0.47-0.70).
2. A seasonal pattern of *S. suis* serotype 2 meningitis was documented in the North Central Viet Nam, but not in Southern Viet Nam.
3. I identified risk factors for *S. suis* infection which can be addressed in health education programs targeted at individuals and communities at risk, focusing on skin protection for those in direct contact with pigs or pork and avoiding eating raw or under-cooked pig products.
4. Human carriage of *S. suis* serotype 2 was not found in the Vietnamese population.

I also propose following my thesis future directions in *S. suis* infection research in Viet Nam:

1. In order to prevent human *S. suis* infection, we should conduct health education programs focused on the important risk factors at communities at risk, such as Thua Thien – Hue province in Central Viet Nam or Soc Trang and Kien Giang provinces in Mekong River Delta.
2. Septic shock in *S. suis* infection was rarely reported in southern Viet Nam, but it was not an uncommon manifestation in the north of the country. Are there any differences in host or bacterial genetics, immune status, exposure factors,

or in treatment delay between septic shock patients and meningitis patients? A study focused on comparison of these characteristics as well as comparing virulence factors of the strains isolated in the north with the strains isolated in the south, needs to be done.

3. Seventy percent of patients were older than 45 years and the incidence rate increased with the incremental of age. Why is *S. suis* infection more common in middle-aged and elderly persons? Does the exposure to pigs peak in this age group or are there decreases in the immune status in the elderly or is the presence of underlying diseases important in increasing the susceptibility to *S. suis* infection in this group?
4. Investigating the prevalence of *S. suis* contamination in food items, such as fresh blood, intestines and uterus, in the restaurants and wet markets could provide support for the hypothesis of local eating habits as a risk factor for infection.
5. Isolation and identification of *S. suis* are not available in every provincial hospital in Viet Nam. A diagnostic algorithm for *S. suis* meningitis based on clinical manifestations and CSF parameters (without the need of bacterial identification) would be useful. In addition, we need to enhance the capacity in bacteriology to establish a diagnosis of *S. suis* infection and to monitor the incidence of disease and the antimicrobial susceptibility of the isolates.

Bibliography

Afifi, S., M. S. Karsany, M. Wasfy, et al. (2009). "Laboratory-based surveillance for patients with acute meningitis in Sudan, 2004-2005." Eur J Clin Microbiol Infect Dis **28**(5): 429-35.

Al-Tawfiq, J. A. and A. Abukhamsin (2009). "Burden and etiology of community-acquired bacterial meningitis in a hospital in Eastern Saudi Arabia: 1993-2005." Med Sci Monit **15**(2): PI10-14.

Allen, A. G., H. Lindsay, D. Seilly, et al. (2004). "Identification and characterisation of hyaluronate lyase from Streptococcus suis." Microb Pathog **36**(6): 327-35.

Anh DD, Kilgore PE, Kennedy WA, et al. (2006). "*Haemophilus influenzae* type b meningitis among children in Hanoi, Vietnam: epidemiologic patterns and estimates of *H. Influenzae* type b disease burden." Am J Trop Med Hyg **74**(3): 509-15.

Anh, D. D., P. E. Kilgore, M. P. Slack, et al. (2009). "Surveillance of pneumococcal-associated disease among hospitalized children in Khanh Hoa Province, Vietnam." Clin Infect Dis **48 Suppl 2**: S57-64.

Arends, J. P. and H. C. Zanen (1988). "Meningitis caused by Streptococcus suis in humans." Rev Infect Dis **10**(1): 131-7.

Aronin, S. I., P. Peduzzi and V. J. Quagliarello (1998). "Community-acquired bacterial meningitis: risk stratification for adverse clinical outcome and effect of antibiotic timing." Ann Intern Med **129**(11): 862-9.

Barnett, A. G. and A. J. Dobson (2010). Analysing Seasonal Health Data (Statistics for Biology and Health), Springer.

Beld, M., R. Minnaar, J. Weel, et al. (2004). "Highly sensitive assay for detection of enterovirus in clinical specimens by reverse transcription-PCR with an armored RNA internal control." J Clin Microbiol **42**(7): 3059-64.

Boisson, C., s. Arnaud, R. Vialet, et al. (1999). "Severe community-acquired meningitis." Crit Care **3**(4): R55-R65.

Brisebois, L. M., R. Charlebois, R. Higgins, et al. (1990). "Prevalence of Streptococcus suis in four to eight week old clinically healthy piglets." Can J Vet Res **54**(1): 174-7.

Brousseau, R., J. E. Hill, G. Prefontaine, et al. (2001). "Streptococcus suis serotypes characterized by analysis of chaperonin 60 gene sequences." Appl Environ Microbiol **67**(10): 4828-33.

Busque, P., R. Higgins, F. Caya, et al. (1997). "Immunization of pigs against Streptococcus suis serotype 2 infection using a live avirulent strain." Can J Vet Res **61**(4): 275-9.

Cam, B. V., L. Fonsmark, N. B. Hue, et al. (2001). "Prospective case-control study of encephalopathy in children with dengue hemorrhagic fever." Am J Trop Med Hyg **65**(6): 848-51.

Campagne, G., A. Schuchat, S. Djibo, et al. (1999). "Epidemiology of bacterial meningitis in Niamey, Niger, 1981-96." Bull World Health Organ **77**(6): 499-508.

Cardosa, M. J., S. M. Wang, M. S. Sum, et al. (2002). "Antibodies against prM protein distinguish between previous infection with dengue and Japanese encephalitis viruses." BMC Microbiol **2**: 9.

Casas, I., A. Tenorio, F. de Ory, et al. (1996). "Detection of both herpes simplex and varicella-zoster viruses in cerebrospinal fluid from patients with encephalitis." J Med Virol **50**(1): 82-92.

Chan, Y. C., A. Wilder-Smith, B. K. Ong, et al. (2002). "Adult community acquired bacterial meningitis in a Singaporean teaching hospital. A seven-year overview (1993-2000)." Singapore Med J **43**(12): 632-6.

Chau, P. Y., C. Y. Huang and R. Kay (1983). "Streptococcus suis meningitis. An important underdiagnosed disease in Hong Kong." Med J Aust **1**(9): 414-6, 417.

Chau, T. T., N. H. Mai, N. H. Phu, et al. (2010). "A prospective descriptive study of cryptococcal meningitis in HIV uninfected patients in Vietnam - high prevalence of Cryptococcus neoformans var grubii in the absence of underlying disease." BMC Infect Dis **10**: 199.

Chen, C., J. Tang, W. Dong, et al. (2007). "A glimpse of streptococcal toxic shock syndrome from comparative genomics of S. suis 2 Chinese isolates." PLoS ONE **2**(3): e315.

Cho, H. K., H. Lee, J. H. Kang, et al. (2010). "The causative organisms of bacterial meningitis in Korean children in 1996-2005." J Korean Med Sci **25**(6): 895-9.

Clifton-Hadley, F. A., T. J. Alexander and M. R. Enright (1986). "Monitoring herds for Streptococcus suis type 2: chance contamination of slaughter pigs." Vet Rec **118**(10): 274.

Clifton-Hadley, F. A. and M. R. Enright (1984). "Factors affecting the survival of Streptococcus suis type 2." Vet Rec **114**(24): 584-6.

CLSI (2010). Performance Standards for Antimicrobial Susceptibility Testing.

Corless, C. E., M. Guiver, R. Borrow, et al. (2001). "Simultaneous detection of Neisseria meningitidis, Haemophilus influenzae, and Streptococcus pneumoniae in suspected cases of meningitis and septicemia using real-time PCR." J Clin Microbiol **39**(4): 1553-8.

CP&HCSC (2010). The 2009 Viet Nam population and housing census: completed results. Ha Noi, Statistical Publishing House.

Dawson, K. G., J. C. Emerson and J. L. Burns (1999). "Fifteen years of experience with bacterial meningitis." Pediatr Infect Dis J **18**(9): 816-22.

de Moor, C. (1963). "Septicaemia infection in pigs, caused by haemolytic streptococci of new Lancefield groups designated R, S and T." Antonie van Leeuwenhoek Journal of Microbiology and Serology **29**: 272-80.

Durand, M. L., S. B. Calderwood, D. J. Weber, et al. (1993). "Acute bacterial meningitis in adults. A review of 493 episodes." N Engl J Med **328**(1): 21-8.

Elliott, S. D., F. Clifton-Hadley and J. Tai (1980). "Streptococcal infection in young pigs. V. An immunogenic polysaccharide from Streptococcus suis type 2 with particular reference to vaccination against streptococcal meningitis in pigs." J Hyg (Lond) **85**(2): 275-85.

Elliott, S. D., M. McCarty and R. C. Lancefield (1977). "Teichoic acids of group D streptococci with special reference to strains from pig meningitis (Streptococcus suis)." J Exp Med **145**(3): 490-9.

Elliott, S. D. and J. Y. Tai (1978). "The type-specific polysaccharides of Streptococcus suis." J Exp Med **148**(6): 1699-704.

Esgleas, M., Y. Li, M. A. Hancock, et al. (2008). "Isolation and characterization of alpha-enolase, a novel fibronectin-binding protein from Streptococcus suis." Microbiology **154**(Pt 9): 2668-79.

Feng, W., S. M. Laster, M. Tompkins, et al. (2001). "In utero infection by porcine reproductive and respiratory syndrome virus is sufficient to increase susceptibility of piglets to challenge by Streptococcus suis type II." J Virol **75**(10): 4889-95.

Fittipaldi, N., T. Collis, B. Prothero, et al. (2009). "Streptococcus suis meningitis, Hawaii." Emerg Infect Dis **15**(12): 2067-9.

Flores-Cordero, J. M., R. Amaya-Villar, M. D. Rincon-Ferrari, et al. (2003). "Acute community-acquired bacterial meningitis in adults admitted to the intensive care unit: clinical manifestations, management and prognostic factors." Intensive Care Med **29**(11): 1967-73.

Gallagher, F. (2001). "Streptococcus infection and splenectomy." Lancet **357**(9262): 1129-30.

Ganiem, A. R., I. Parwati, R. Wisaksana, et al. (2009). "The effect of HIV infection on adult meningitis in Indonesia: a prospective cohort study." Aids **23**(17): 2309-16.

Gjini, A. B., J. M. Stuart, D. A. Lawlor, et al. (2006). "Changing epidemiology of bacterial meningitis among adults in England and Wales 1991-2002." Epidemiol Infect **134**(3): 567-9.

Glaser, C. A., S. Honarmand, L. J. Anderson, et al. (2006). "Beyond viruses: clinical profiles and etiologies associated with encephalitis." Clin Infect Dis **43**(12): 1565-77.

Gottschalk, M. (2004). "Porcine *Streptococcus suis* strains as potential sources of infections in humans: an underdiagnosed problem in North America?" Journal of Swine Health and Production **12**(4): 197-9.

Gottschalk, M., R. Higgins, M. Jacques, et al. (1989). "Description of 14 new capsular types of Streptococcus suis." J Clin Microbiol **27**(12): 2633-6.

Gottschalk, M., A. Lebrun, H. Wisselink, et al. (1998). "Production of virulence-related proteins by Canadian strains of Streptococcus suis capsular type 2." Can J Vet Res **62**(1): 75-9.

Gottschalk, M. and M. Segura (2000). "The pathogenesis of the meningitis caused by Streptococcus suis: the unresolved questions." Vet Microbiol **76**(3): 259-72.

Gottschalk, M., M. Segura and J. Xu (2007). "Streptococcus suis infections in humans: the Chinese experience and the situation in North America." Anim Health Res Rev **8**(1): 29-45.

Gottschalk, M., J. Xu, C. Calzas, et al. (2010). "Streptococcus suis: a new emerging or an old neglected zoonotic pathogen?" Future Microbiol **5**: 371-91.

Granerod, J., H. E. Ambrose, N. W. Davies, et al. (2010). "Causes of encephalitis and differences in their clinical presentations in England: a multicentre, population-based prospective study." Lancet Infect Dis **10**(12): 835-44.

Greger, M. (2007). "The human/animal interface: emergence and resurgence of zoonotic infectious diseases." Crit Rev Microbiol **33**(4): 243-99.

GSO (2009). Statistical Yearbook of Viet Nam 2008. Ha Noi, Statistical Publishing House.

GSO (2010). Statistical Yearbook of Viet Nam 2009. Ha Noi, Statistical Publishing House.

Gurley, E. S., M. J. Hossain, S. P. Montgomery, et al. (2009). "Etiologies of bacterial meningitis in Bangladesh: results from a hospital-based study." Am J Trop Med Hyg **81**(3): 475-83.

Ha, D., V. Huong, H. Loan, et al. (1994). "Current situation of Japanese encephaltis in the South of Viet Nam, 1976-1992." Trop. Med.: 202-214.

Haleis, A., M. Alfa, M. Gottschalk, et al. (2009). "Meningitis caused by Streptococcus suis serotype 14, North America." Emerg Infect Dis **15**(2): 350-2.

Han, D. U., C. Choi, H. J. Ham, et al. (2001). "Prevalence, capsular type and antimicrobial susceptibility of Streptococcus suis isolated from slaughter pigs in Korea." Can J Vet Res **65**(3): 151-5.

Harrell, F. (2001). Regression Modeling Strategies With Applications to Linear Models, Logistic Regression, and Survival Analysis. New York, Springer.

Hendarto, S. K. and S. R. Hadinegoro (1992). "Dengue encephalopathy." Acta Paediatr Jpn **34**(3): 350-7.

Higgins, R. and M. Gottschalk (1990). "An update on Streptococcus suis identification." J Vet Diagn Invest **2**(3): 249-52.

Higgins, R. and M. Gottschalk (2001). "Distribution of Streptococcus suis capsular types in 2000." Can Vet J **42**(3): 223.

Higgins, R., M. Gottschalk, M. Boudreau, et al. (1995). "Description of six new capsular types (29-34) of Streptococcus suis." J Vet Diagn Invest **7**(3): 405-6.

Hill, J. E., M. Gottschalk, R. Brousseau, et al. (2005). "Biochemical analysis, cpn60 and 16S rDNA sequence data indicate that Streptococcus suis serotypes 32 and 34, isolated from pigs, are Streptococcus orisratti." Vet Microbiol **107**(1-2): 63-9.

Hoa, N. T., T. T. Chieu, T. T. Nga, et al. (2011). "Slaughterhouse Pigs Are a Major Reservoir of Streptococcus suis Serotype 2 Capable of Causing Human Infection in Southern Vietnam." PLoS ONE **6**(3): e17943.

Hoa, N. T., T. T. Chieu, H. D. Nghia, et al. (2011). "The antimicrobial resistance patterns and associated determinants in Streptococcus suis isolated from humans in southern Vietnam, 1997-2008." BMC Infect Dis **11**: 6.

Holden, M. T., H. Hauser, M. Sanders, et al. (2009). "Rapid evolution of virulence and drug resistance in the emerging zoonotic pathogen Streptococcus suis." PLoS One **4**(7): e6072.

Holt, M. E., M. R. Enright and T. J. Alexander (1990). "Immunisation of pigs with killed cultures of Streptococcus suis type 2." Res Vet Sci **48**(1): 23-7.

Huang, Y. T., L. J. Teng, S. W. Ho, et al. (2005). "Streptococcus suis infection." J Microbiol Immunol Infect **38**(5): 306-13.

Hui, A. C., K. C. Ng, P. Y. Tong, et al. (2005). "Bacterial meningitis in Hong Kong: 10-years' experience." Clin Neurol Neurosurg **107**(5): 366-70.

Huppatz, C., D. N. Durrheim, C. Levi, et al. (2009). "Etiology of encephalitis in Australia, 1990-2007." Emerg Infect Dis **15**(9): 1359-65.

Ihekwaba, U. K., G. Kudesia and M. W. McKendrick (2008). "Clinical features of viral meningitis in adults: significant differences in cerebrospinal fluid findings among herpes simplex virus, varicella zoster virus, and enterovirus infections." Clin Infect Dis **47**(6): 783-9.

Ip, M., K. S. Fung, F. Chi, et al. (2007). "Streptococcus suis in Hong Kong." Diagn Microbiol Infect Dis **57**(1): 15-20.

Jackson, S., A. Mullings, F. Bennett, et al. (2008). "Dengue Infection in Patients Presenting with Neurological Manifestations in a Dengue Endemic Population." West Indian Med J **57**(4): 473-6.

Jacobs, A. A., P. L. Loeffen, A. J. van den Berg, et al. (1994). "Identification, purification, and characterization of a thiol-activated hemolysin (suilysin) of Streptococcus suis." Infect Immun **62**(5): 1742-8.

Jacobs, A. A., A. J. van den Berg and P. L. Loeffen (1996). "Protection of experimentally infected pigs by suilysin, the thiol-activated haemolysin of Streptococcus suis." Vet Rec **139**(10): 225-8.

Jerome, K. R., M. L. Huang, A. Wald, et al. (2002). "Quantitative stability of DNA after extended storage of clinical specimens as determined by real-time PCR." J Clin Microbiol **40**(7): 2609-11.

Kataoka, Y., T. Yoshida and T. Sawada (2000). "A 10-year survey of antimicrobial susceptibility of streptococcus suis isolates from swine in Japan." J Vet Med Sci **62**(10): 1053-7.

Kay, R. (1991). "The site of the lesion causing hearing loss in bacterial meningitis: a study of experimental streptococcal meningitis in guinea-pigs." Neuropathol Appl Neurobiol **17**(6): 485-93.

Kay, R., A. F. Cheng and C. Y. Tse (1995). "Streptococcus suis infection in Hong Kong." Qjm **88**(1): 39-47.

Kerdsin, A., K. Oishi, S. Sripakdee, et al. (2009). "Clonal dissemination of human isolates of Streptococcus suis serotype 14 in Thailand." J Med Microbiol **58**(Pt 11): 1508-13.

Khadthasrima, N., T. Hannwong, P. Thammawitjaya, et al. (2009). "Human Streptococcus suis outbreak in Phayao province, Thailand, 2007." Retrieved 25 March, 2010, from http://osirjournal.net/upload/files/Streptococcus_suis.pdf.

Khwannimit, B., P. Chayakul and A. Geater (2004). "Acute bacterial meningitis in adults: a 20 year review." Southeast Asian J Trop Med Public Health **35**(4): 886-92.

Kopic, J., M. T. Paradzik and N. Pandak (2002). "Streptococcus suis infection as a cause of severe illness: 2 cases from Croatia." Scand J Infect Dis **34**(9): 683-4.

Koskiniemi, M., H. Piiparinen, L. Mannonen, et al. (1996). "Herpes encephalitis is a disease of middle aged and elderly people: polymerase chain reaction for detection of herpes simplex virus in the CSF of 516 patients with encephalitis. The Study Group." J Neurol Neurosurg Psychiatry **60**(2): 174-8.

Kumar, P., R. Kumar, K. L. Srivastava, et al. (2005). "Protective role of BCG vaccination against tuberculous meningitis in Indian children: a reappraisal." Natl Med J India **18**(1): 7-11.

Lakkitjaroen, N., S. Kaewmongkol, P. Metheenukul, et al. (2011). "Prevalence and Antimicrobial susceptibility of *Streptococcus suis* isolated from slaughter pigs in northern Thailand." Kasetsart J. (Nat. Sci.) **45**: 78-83.

Lamont, M. H., P. T. Edwards and R. S. Windsor (1980). "Streptococcal meningitis in pigs: results of a five-year survey." Vet Rec **107**(20): 467-9.

Le, T., T. T. Hong Chau, N. T. Kim Cuc, et al. (2010). "AIDS-associated Cryptococcus neoformans and Penicillium marneffei coinfection: a therapeutic dilemma in resource-limited settings." Clin Infect Dis **51**(9): e65-8.

Le, V. T., T. Q. Phan, Q. H. Do, et al. (2010). "Viral etiology of encephalitis in children in southern Vietnam: results of a one-year prospective descriptive study." PLoS Negl Trop Dis **4**(10): e854.

Le, X., M. Pham and H. Pham (2007). "Study of eosinophilic meningitis in Ho Chi Minh city, Vietnam." Southeast Asian J Trop Med Public Health **38**(suppl 1): 47-50.

Lee, B. E., R. Chawla, J. M. Langley, et al. (2006). "Paediatric Investigators Collaborative Network on Infections in Canada (PICNIC) study of aseptic meningitis." BMC Infect Dis **6**: 68.

Lee, G. T., C. Y. Chiu, B. L. Haller, et al. (2008). "Streptococcus suis meningitis, United States." Emerg Infect Dis **14**(1): 183-5.

Leelarasamee, A., C. Nilakul, S. Tien-Grim, et al. (1997). "Streptococcus suis toxic-shock syndrome and meningitis." J Med Assoc Thai **80**(1): 63-8.

Li, M., C. Wang, Y. Feng, et al. (2008). "SalK/SalR, a two-component signal transduction system, is essential for full virulence of highly invasive Streptococcus suis serotype 2." PLoS ONE **3**(5): e2080.

Li, W., Z. Shi, M. Yu, et al. (2005). "Bats are natural reservoirs of SARS-like coronaviruses." Science **310**(5748): 676-9.

Li, Y., M. Gottschalk, M. Esgleas, et al. (2007). "Immunization with recombinant Sao protein confers protection against Streptococcus suis infection." Clin Vaccine Immunol **14**(8): 937-43.

Li, Y., G. Martinez, M. Gottschalk, et al. (2006). "Identification of a surface protein of Streptococcus suis and evaluation of its immunogenic and protective capacity in pigs." Infect Immun **74**(1): 305-12.

Lo Re, V., 3rd and S. J. Gluckman (2003). "Eosinophilic meningitis." Am J Med **114**(3): 217-23.

Lu, C. H., C. R. Huang, W. N. Chang, et al. (2002). "Community-acquired bacterial meningitis in adults: the epidemiology, timing of appropriate antimicrobial therapy, and prognostic factors." Clin Neurol Neurosurg **104**(4): 352-8.

Lun, Z. R., Q. P. Wang, X. G. Chen, et al. (2007). "Streptococcus suis: an emerging zoonotic pathogen." Lancet Infect Dis **7**(3): 201-9.

Ma, E., P. H. Chung, T. So, et al. (2008). "Streptococcus suis infection in Hong Kong: an emerging infectious disease?" Epidemiol Infect: 1-7.

Ma, Y., Y. Feng, D. Liu, et al. (2009). "Avian influenza virus, Streptococcus suis serotype 2, severe acute respiratory syndrome-coronavirus and beyond: molecular epidemiology, ecology and the situation in China." Philos Trans R Soc Lond B Biol Sci **364**(1530): 2725-37.

Madsen, L. W., B. Nielsen, B. Aalbaek, et al. (2001). "Experimental infection of conventional pigs with Streptococcus suis serotype 2 by aerosolic exposure." Acta Vet Scand **42**(2): 303-6.

Mai, N. T., N. T. Hoa, T. V. Nga, et al. (2008). "Streptococcus suis meningitis in adults in Vietnam." Clin Infect Dis **46**(5): 659-67.

Mailles, A. and J. P. Stahl (2009). "Infectious encephalitis in france in 2007: a national prospective study." Clin Infect Dis **49**(12): 1838-47.

Marais, S., G. Thwaites, J. F. Schoeman, et al. (2010). "Tuberculous meningitis: a uniform case definition for use in clinical research." Lancet Infect Dis **10**(11): 803-12.

Marie, J., H. Morvan, F. Berthelot-Herault, et al. (2002). "Antimicrobial susceptibility of Streptococcus suis isolated from swine in France and from humans in different countries between 1996 and 2000." J Antimicrob Chemother **50**(2): 201-9.

Marois, C., L. Le Devendec, M. Gottschalk, et al. (2007). "Detection and molecular typing of Streptococcus suis in tonsils from live pigs in France." Can J Vet Res **71**(1): 14-22.

Martinez, G., J. Harel, S. Lacouture, et al. (2002). "Genetic diversity of Streptococcus suis serotypes 2 and 1/2 isolates recovered from carrier pigs in closed herds." Can J Vet Res **66**(4): 240-8.

MoH (2008). Report of Viet Nam Tuberculosis Control Programme.

MoH (2009). Report of Viet Nam Tuberculosis Control Programme.

MoH. (2010). "Viet Nam HIV and AIDS data." Retrieved Mar 07, 2011, from http://www.vaac.gov.vn/Download.aspx/23D6EA077799489E8BEF4A82A7F71356/1/3Phan_tich_tinh_hinh_dich_nam_2009_01-04-2010-1.pdf.

Molyneux, E., F. A. Riordan and A. Walsh (2006). "Acute bacterial meningitis in children presenting to the Royal Liverpool Children's Hospital, Liverpool, UK and the Queen Elizabeth Central Hospital in Blantyre, Malawi: a world of difference." Ann Trop Paediatr **26**(1): 29-37.

Muangchana, C., S. Chunsuttiwat, S. Rerks-Ngarm, et al. (2009). "Bacterial meningitis incidence in Thai children estimated by a rapid assessment tool (RAT)." Southeast Asian J Trop Med Public Health **40**(3): 553-62.

Mwaniki, C. G., I. D. Robertson and D. J. Hampson (1994). "The prevalence of Streptococcus suis type 2 in Western Australian piggeries." Aust Vet J **71**(11): 385-6.

Navacharoen, N., V. Chantharochavong, C. Hanprasertpong, et al. (2009). "Hearing and vestibular loss in Streptococcus suis infection from swine and traditional raw pork exposure in northern Thailand." J Laryngol Otol **123**(8): 857-62.

Nga, T. V. T., H. D. Nghia, L. T. P. Tu, et al. (2011). "Real-time PCR for detection of Streptococcus suis serotype 2 in cerebrospinal fluid of human patients with meningitis." Diagn Microbiol Infect Dis **70**(4): 461-7.

Nghia, H. D., N. T. Hoa, D. Linh le, et al. (2008). "Human case of Streptococcus suis serotype 16 infection." Emerg Infect Dis **14**(1): 155-7.

Nguyen, T. H., T. H. Tran, G. Thwaites, et al. (2007). "Dexamethasone in Vietnamese adolescents and adults with bacterial meningitis." N Engl J Med **357**(24): 2431-40.

Nudelman, Y. and A. R. Tunkel (2009). "Bacterial meningitis: epidemiology, pathogenesis and management update." Drugs **69**(18): 2577-96.

Ooi, M. H., S. C. Wong, Y. Podin, et al. (2007). "Human enterovirus 71 disease in Sarawak, Malaysia: a prospective clinical, virological, and molecular epidemiological study." Clin Infect Dis **44**(5): 646-56.

Park, B. J., K. A. Wannemuehler, B. J. Marston, et al. (2009). "Estimation of the current global burden of cryptococcal meningitis among persons living with HIV/AIDS." Aids **23**(4): 525-30.

Peltola, H. (2000). "Worldwide Haemophilus influenzae type b disease at the beginning of the 21st century: global analysis of the disease burden 25 years after the use of the polysaccharide vaccine and a decade after the advent of conjugates." Clin Microbiol Rev **13**(2): 302-17.

Perch, B., P. Kristjansen and K. Skadhauge (1968). "Group R streptococci pathogenic for man. Two cases of meningitis and one fatal case of sepsis." Acta Pathol Microbiol Scand **74**(1): 69-76.

Reams, R. Y., L. T. Glickman, D. D. Harrington, et al. (1993). "Streptococcus suis infection in swine: a retrospective study of 256 cases. Part I. Epidemiologic factors and antibiotic susceptibility patterns." J Vet Diagn Invest **5**(3): 363-7.

Robertson, I. D. and D. K. Blackmore (1989). "Occupational exposure to Streptococcus suis type 2." Epidemiol Infect **103**(1): 157-64.

Robertson, I. D. and D. K. Blackmore (1989). "Prevalence of Streptococcus suis types 1 and 2 in domestic pigs in Australia and New Zealand." Vet Rec **124**(15): 391-4.

Rusmeechan, S. and P. Sribusara (2008). "Streptococcus suis meningitis: the newest serious infectious disease." J Med Assoc Thai **91**(5): 654-8.

Salmon, S. A., J. L. Watts, C. A. Case, et al. (1995). "Comparison of MICs of ceftiofur and other antimicrobial agents against bacterial pathogens of swine from the United States, Canada, and Denmark." J Clin Microbiol **33**(9): 2435-44.

Scarborough, M., S. B. Gordon, C. J. Whitty, et al. (2007). "Corticosteroids for bacterial meningitis in adults in sub-Saharan Africa." N Engl J Med **357**(24): 2441-50.

Scheltinga, S. A., K. E. Templeton, M. F. Beersma, et al. (2005). "Diagnosis of human metapneumovirus and rhinovirus in patients with respiratory tract infections by an internally controlled multiplex real-time RNA PCR." J Clin Virol **33**(4): 306-11.

Shetty, S., A. L. Cohen, K. Edmond, et al. (2010). "A systematic review and critical evaluation of invasive Haemophilus influenzae type B disease burden studies in Asia from the last decade: lessons learned for invasive bacterial disease surveillance." Pediatr Infect Dis J **29**(7): 653-61.

Smith, H. E., M. Damman, J. van der Velde, et al. (1999). "Identification and characterization of the cps locus of Streptococcus suis serotype 2: the capsule protects against phagocytosis and is an important virulence factor." Infect Immun **67**(4): 1750-6.

Smith, T. C., A. W. Capuano, B. Boese, et al. (2008). "Exposure to Streptococcus suis among US swine workers." Emerg Infect Dis **14**(12): 1925-7.

Soares, C. N., M. J. Cabral-Castro, J. M. Peralta, et al. (2011). "Review of the etiologies of viral meningitis and encephalitis in a dengue endemic region." J Neurol Sci.

Solomon, T. (2004). "Flavivirus encephalitis." N Engl J Med **351**(4): 370-8.

Solomon, T., N. M. Dung, R. Kneen, et al. (2000). "Japanese encephalitis." J Neurol Neurosurg Psychiatry **68**(4): 405-15.

Solomon, T., N. M. Dung, D. W. Vaughn, et al. (2000). "Neurological manifestations of dengue infection." Lancet **355**(9209): 1053-9.

Song, J. H., S. I. Jung, K. S. Ko, et al. (2004). "High prevalence of antimicrobial resistance among clinical Streptococcus pneumoniae isolates in Asia (an ANSORP study)." Antimicrob Agents Chemother **48**(6): 2101-7.

Srey, V. H., H. Sadones, S. Ong, et al. (2002). "Etiology of encephalitis syndrome among hospitalized children and adults in Takeo, Cambodia, 1999-2000." Am J Trop Med Hyg **66**(2): 200-7.

Sriskandan, S. and J. D. Slater (2006). "Invasive disease and toxic shock due to zoonotic Streptococcus suis: an emerging infection in the East?" PLoS Med **3**(5): e187.

Staats, J. J., I. Feder, O. Okwumabua, et al. (1997). "Streptococcus suis: past and present." Vet Res Commun **21**(6): 381-407.

Stephens, D. S., B. Greenwood and P. Brandtzaeg (2007). "Epidemic meningitis, meningococcaemia, and Neisseria meningitidis." Lancet **369**(9580): 2196-210.

Stevens, D. L. (1995). "Streptococcal toxic-shock syndrome: spectrum of disease, pathogenesis, and new concepts in treatment." Emerg Infect Dis **1**(3): 69-78.

Strangmann, E., H. Froleke and K. P. Kohse (2002). "Septic shock caused by Streptococcus suis: case report and investigation of a risk group." Int J Hyg Environ Health **205**(5): 385-92.

Suankratay, C., P. Intalapaporn, P. Nunthapisud, et al. (2004). "Streptococcus suis meningitis in Thailand." Southeast Asian J Trop Med Public Health **35**(4): 868-76.

Tang, J., C. Wang, Y. Feng, et al. (2006). "Streptococcal toxic shock syndrome caused by Streptococcus suis serotype 2." PLoS Med **3**(5): e151.

Tang, L. M., S. T. Chen, W. C. Hsu, et al. (1999). "Acute bacterial meningitis in adults: a hospital-based epidemiological study." Qjm **92**(12): 719-25.

Tang, Y. W., M. J. Espy, D. H. Persing, et al. (1997). "Molecular evidence and clinical significance of herpesvirus coinfection in the central nervous system." J Clin Microbiol **35**(11): 2869-72.

Tansuphasawadikul, S., P. N. Amornkul, C. Tanchanpong, et al. (1999). "Clinical presentation of hospitalized adult patients with HIV infection and AIDS in Bangkok, Thailand." J Acquir Immune Defic Syndr **21**(4): 326-32.

Tarradas, C., I. Luque, D. de Andres, et al. (2001). "Epidemiological relationship of human and swine Streptococcus suis isolates." J Vet Med B Infect Dis Vet Public Health **48**(5): 347-55.

Thanawongnuwech, R., G. B. Brown, P. G. Halbur, et al. (2000). "Pathogenesis of porcine reproductive and respiratory syndrome virus-induced increase in susceptibility to Streptococcus suis infection." Vet Pathol **37**(2): 143-52.

Thwaites, G., T. T. Chau, N. T. Mai, et al. (2000). "Tuberculous meningitis." J Neurol Neurosurg Psychiatry **68**(3): 289-99.

Thwaites, G. E., T. T. Chau, K. Stepniewska, et al. (2002). "Diagnosis of adult tuberculous meningitis by use of clinical and laboratory features." Lancet **360**(9342): 1287-92.

Thwaites, G. E., D. B. Nguyen, H. D. Nguyen, et al. (2004). "Dexamethasone for the treatment of tuberculous meningitis in adolescents and adults." N Engl J Med **351**(17): 1741-51.

Tramontana, A. R., M. Graham, V. Sinickas, et al. (2008). "An Australian case of Streptococcus suis toxic shock syndrome associated with occupational exposure to animal carcasses." Med J Aust **188**(9): 538-9.

Tran, T. T., Q. T. Le, T. N. Tran, et al. (1998). "The etiology of bacterial pneumonia and meningitis in Vietnam." Pediatr Infect Dis J **17**(9 Suppl): S192-4.

Trottier, S., R. Higgins, G. Brochu, et al. (1991). "A case of human endocarditis due to Streptococcus suis in North America." Rev Infect Dis **13**(6): 1251-2.

Trunz, B. B., P. Fine and C. Dye (2006). "Effect of BCG vaccination on childhood tuberculous meningitis and miliary tuberculosis worldwide: a meta-analysis and assessment of cost-effectiveness." Lancet **367**(9517): 1173-80.

Tu, P. V., N. T. Thao, D. Perera, et al. (2007). "Epidemiologic and virologic investigation of hand, foot, and mouth disease, southern Vietnam, 2005." Emerg Infect Dis **13**(11): 1733-41.

van de Beek, D., J. de Gans, L. Spanjaard, et al. (2004). "Clinical features and prognostic factors in adults with bacterial meningitis." N Engl J Med **351**(18): 1849-59.

van de Beek, D., L. Spanjaard and J. de Gans (2008). "Streptococcus suis meningitis in the Netherlands." J Infect.

van Deuren, M., P. Brandtzaeg and J. W. van der Meer (2000). "Update on meningococcal disease with emphasis on pathogenesis and clinical management." Clin Microbiol Rev **13**(1): 144-66, table of contents.

van Doornum, G. J., J. Guldemeester, A. D. Osterhaus, et al. (2003). "Diagnosing herpesvirus infections by real-time amplification and rapid culture." J Clin Microbiol **41**(2): 576-80.

Varatharaj, A. (2010). "Encephalitis in the clinical spectrum of dengue infection." Neurol India **58**(4): 585-91.

Vecht, U., H. J. Wisselink, M. L. Jellema, et al. (1991). "Identification of two proteins associated with virulence of Streptococcus suis type 2." Infect Immun **59**(9): 3156-62.

Vianello, F., F. Barbaro, P. Cogo, et al. (2008). "Co-infection with Mycoplasma pneumoniae and human herpesvirus 6 (HHV-6) in an immunocompetent child with meningoencephalitis: a random association?" Infection **36**(2): 174-6.

Vilaichone, R. K., V. Mahachai and P. Nunthapisud (2000). "Streptococcus suis peritonitis: case report." J Med Assoc Thai **83**(10): 1274-7.

Vilaichone, R. K., W. Vilaichone, P. Nunthapisud, et al. (2002). "Streptococcus suis infection in Thailand." J Med Assoc Thai **85 Suppl 1**: S109-17.

Walsh, B., A. E. Williams and J. Satsangi (1992). "*Streptococcus suis* type 2: pathogenesis and clinical disease." Review in Medical Microbiology **3**: 65-71.

Wangkaew, S., R. Chaiwarith, P. Tharavichitkul, et al. (2006). "Streptococcus suis infection: a series of 41 cases from Chiang Mai University Hospital." J Infect **52**(6): 455-60.

Wangsomboonsiri, W., T. Luksananun, S. Saksornchai, et al. (2008). "Streptococcus suis infection and risk factors for mortality." J Infect **57**(5): 392-6.

Weinberg, A., K. C. Bloch, S. Li, et al. (2005). "Dual infections of the central nervous system with Epstein-Barr virus." J Infect Dis **191**(2): 234-7.

Weisfelt, M., D. van de Beek, L. Spanjaard, et al. (2006). "Clinical features, complications, and outcome in adults with pneumococcal meningitis: a prospective case series." Lancet Neurol **5**(2): 123-9.

Wenger, J. D., A. W. Hightower, R. R. Facklam, et al. (1990). "Bacterial meningitis in the United States, 1986: report of a multistate surveillance study. The Bacterial Meningitis Study Group." J Infect Dis **162**(6): 1316-23.

Wertheim, H. F., H. D. Nghia, W. Taylor, et al. (2009). "Streptococcus suis: an emerging human pathogen." Clin Infect Dis **48**(5): 617-25.

Wertheim, H. F., H. N. Nguyen, W. Taylor, et al. (2009). "Streptococcus suis, an important cause of adult bacterial meningitis in northern Vietnam." PLoS One **4**(6): e5973.

WHO (2003). WHO-recomended standards for surveillance of selected vaccine-preventable diseases. Geneva, Switzerland, World Health Organisation.

WHO (2008). The global burden of diseases: 2004 update. Geneva, Switzerland, World Health Organization.

WHO. (2009). "Dengue in the Western Pacific Region." Retrieved March 3rd, 2011, from http://www.wpro.who.int/health_topics/dengue/.

WHO (2010). Global tuberculosis control: WHO report 2010. Geneva, Switzerland, World Health Organization.

WHO. (2010). "Viet Nam - National Expenditure on Health." Retrieved Mar 17, 2011, from http://www.who.int/nha/country/vnm/en/.

WHO (2010). World Health Statistics 2010, WHO**:** 177.

Willenburg, K. S., D. E. Sentochnik and R. N. Zadoks (2006). "Human Streptococcus suis meningitis in the United States." N Engl J Med **354**(12): 1325.

Windsor, R. S. and S. D. Elliott (1975). "Streptococcal infection in young pigs. IV. An outbreak of streptococcal meningitis in weaned pigs." J Hyg (Lond) **75**(1): 69-78.

Wisselink, H. J., F. H. Reek, U. Vecht, et al. (1999). "Detection of virulent strains of Streptococcus suis type 2 and highly virulent strains of Streptococcus suis type 1 in tonsillar specimens of pigs by PCR." Vet Microbiol **67**(2): 143-57.

Wisselink, H. J., U. Vecht, N. Stockhofe-Zurwieden, et al. (2001). "Protection of pigs against challenge with virulent Streptococcus suis serotype 2 strains by a muramidase-released protein and extracellular factor vaccine." Vet Rec **148**(15): 473-7.

Wisselink, H. J., K. T. Veldman, C. Van den Eede, et al. (2006). "Quantitative susceptibility of Streptococcus suis strains isolated from diseased pigs in seven European countries to antimicrobial agents licensed in veterinary medicine." Vet Microbiol **113**(1-2): 73-82.

Woolhouse, M. E. and S. Gowtage-Sequeria (2005). "Host range and emerging and reemerging pathogens." Emerg Infect Dis **11**(12): 1842-7.

Xu, M., S. Wang, L. Li, et al. (2010). "Secondary infection with Streptococcus suis serotype 7 increases the virulence of highly pathogenic porcine reproductive and respiratory syndrome virus in pigs." Virol J **7**: 184.

Yamamoto, T. and Y. Nakamura (2000). "A single tube PCR assay for simultaneous amplification of HSV-1/-2, VZV, CMV, HHV-6A/-6B, and EBV DNAs in cerebrospinal fluid from patients with virus-related neurological diseases." J Neurovirol **6**(5): 410-7.

Yen NT, Duffy MR, Hong NM, et al. (2010). "Surveillance for Japanese encephalitis in Vietnam, 1998-2007." Am J Trop Med Hyg **83**(4): 816-19.

Yong, Y. K., H. T. Chong, K. T. Wong, et al. (2008). "Aetiology of viral central nervous system infection, a Malaysian study." Neurology Asia **13**: 65-71.

Yu, H., H. Jing, Z. Chen, et al. (2006). "Human Streptococcus suis outbreak, Sichuan, China." Emerg Infect Dis **12**(6): 914-20.

Yu, H. J., X. C. Liu, S. W. Wang, et al. (2005). "[Matched case-control study for risk factors of human Streptococcus suis infection in Sichuan Province, China]." Zhonghua Liu Xing Bing Xue Za Zhi **26**(9): 636-9.

Zhang, A., B. Chen, X. Mu, et al. (2009). "Identification and characterization of a novel protective antigen, Enolase of Streptococcus suis serotype 2." Vaccine **27**(9): 1348-53.

Zhang, C., Y. Ning, Z. Zhang, et al. (2008). "In vitro antimicrobial susceptibility of Streptococcus suis strains isolated from clinically healthy sows in China." Vet Microbiol **131**: 386-392.

Appendix A

The prospective surveillance study of CNS infection in the Central, Central Highlands and South of Viet Nam (01SS study)

I. Case record forms:

a. Adult patient:

DATA FORM

Study number:

Hospital number:

GENERAL INFORMATION

1. Initials:/__/__/__/ Age: ..... Sex: Male / Female
2. Address ..... Commune:.....
   District: ..... Province: .....
   Rural / Urban
3. Occupation:.....
4. Ethnic groups:.....
5. Date of admission (DD/MM/YYYY): /___/___/_____/
6. Reason of admission:.....

UNDERLYING DISEASES or PAST MEDICAL HISTORY

7. Underlying diseases or past medical history: (Yes: Y; No: N; Unknown: U)

- Diabetes Y / N / U
- Otitis Y / N / U
- Head trauma Y / N / U
- Heart diseases Y / N / U
- Alcoholic Y / N / U
- Splenectomy Y / N / U
- Bacterial meningitis Y / N / U
- Valvular heart diseases Y/N / U
- Others:.....

PIG EXPOSURE

8. In patient's family, is there anybody that has job related to pigs? Y / N / U

- Pig breeder ☐
- Butcher ☐
- Slaughterer ☐
- Meat processor ☐
- Meat transporter ☐
- Pork roaster ☐
- Waiter ☐
- Veterinary inspector ☐

9. Does the patient have any pigs at home? Y / N / U

10. Have his/her pigs had any diseases in the last 4 weeks? Y / N / U

11. If yes, describe:

- Fever ☐
- Arthritis ☐
- Neurological problems ☐
- Cough ☐
- Sudden death ☐
- Miscarriage ☐
- Unknown ☐
- Others: ……

12. Has the patient done any of the work below during the last 2 weeks?

- Bathe the pigs ☐
- Feed pigs ☐
- Clean up the piggery ☐
- Slaughter pigs ☐
- Prepare/handle pig blood ☐
- Prepare/handle raw pork ☐
- Contact with pig's organs ☐
- Cook pork ☐
- Not done ☐
- Unknown ☐
- Others: ……

13. Which dishes did patient take in the last 2 weeks (especially when drinking)?

- Pig's blood curds ☐
- Duck's blood curds ☐
- Undercooked pig's blood ☐
- Pig tonsils or tongue ☐

- Pig stomach or intestines ☐
- Pig uterus ☐
- Not eat ☐
- Unknown ☐

CLINICAL MANIFESTATIONS ON ADMISSION

14. Days of illness: …….days

15. Antimicrobial therapy before admission? Yes/No/Unknown

If yes, could you describe:

……

……

16 Vital signs:

- Pulse: /minute
- Temperature: ^{0}C
- Blood pressure: / mmHg
- Breath rate: /minute

17. Glasgow Coma Scale: E M V =

18. Clinical manifestations:

- Fever Y / N / U
- Chills/rigors Y / N / U
- Headache Y / N / U
- Nausea/vomiting Y / N / U
- Diarrhoea Y / N / U
- Dizziness Y / N / U
- Tinnitus Y / N / U
- Deafness Y / N / U
- Delirium Y / N / U
- Confusion Y / N / U
- Unconscious Y / N / U
- Seizures Y / N / U
- Gastrointestinal haemorrhage Y / N / U
- Other haemorrhages Y / N / U
  ……
  ……
- Acrocyanosis/ Necrosis Y / N / U
- Rash Y / N / U
  ……
  ……
- Jaundice Y / N / U
- Hepatomegaly Y / N / U
- Urine retention Y / N / U
- Meningeal signs Y / N / U
- Hemiplegia Y / N / U
- Paraplegia Y / N / U
- Cranial nerve palsy Y / N / U
- Herpes labial Y / N / U

- Arthritis Y / N / U

  ……

  ……

- Ear drum:

  L Normal/ Abnormal

  R Normal/ Abnormal

- Heart exam:

  Normal/Abnormal

  ……

  ……

- Lung exam:

  Normal/ Abnormal

  ……

  ……

- Skin lesions Y / N / U

  ……

  ……

  ……

  ……

LABORATORY FINDINGS ON ADMISSION

19. CBC

WBC ...../µl (N.....%, L.....%, M.....%, E.....%)

Hb.....g/dl, Hct.....%

Plt...../ µl

20. Liver function tests:

SGOT .....U/l, SGPT.....U/l, Bilirubin $_{total}$ .....µmol/l

21. Renal function tests:

BUN .....mmol/l, Creatinine .....µmol/l

22. CSF:

Date				
Color…				
Opening pressure				
Cell count + WC (N/L) + RC				
Protein				
Glucose (CSF/blood)				
Lactate (CSF/blood)				
Gram stain				
Culture				
PCR				
ELISA				

23. CSF culture and antibiogram:

……

……

……

……

24. Blood culture and antibiogram:

……

……

……

……

25. Chest X-ray: Normal / Abnormal / Not done

……

……

26. Cranial CT Scan/MRI: Normal / Abnormal / Not done

……

……

……

TREATMENT AND OUTCOME

27. Final diagnosis: .....

28. Dexamethasone: Y / N / U

29. Antimicrobial therapy:

……

……

……

……

30. Hearing status on discharge:

- Tinnitus Y / N / U
- Hearing loss Y / N / U
- Deaf (completely) Y / N / U

31. Other sequelae:

……

32. Outcome:

- Survival ☐
- Died ☐
- Transferred ☐
- Unknown ☐

31. Duration of staying in hospital: …… day(s).

b. Paediatric patient:

DATA FORM

Study number:

Hospital number:

GENERAL INFORMATION

1. Initials:/__/__/__/ Age: .....(yrs) .....(months) Sex: Male / Female
2. Address ..... Commune:.....
   District: ..... Province: .....
   Rural / Urban
3. Occupation:.....
4. Ethnic groups:.....
5. Date of admission (DD/MM/YYYY): /___/___/_____/
6. Reason of admission:.....

UNDERLYING DISEASES or PAST MEDICAL HISTORY

7. Underlying diseases or past medical history: (Yes: Y; No: N; Unknown: U)

- Diabetes Y / N / U
- Otitis/ Sinusitis Y / N / U
- Head trauma Y / N / U
- Heart diseases Y / N / U
- Mother had fever on delivery Y / N / U
- Others:.....
- Splenectomy Y / N / U
- Bacterial meningitis Y / N / U
- Valvular heart diseases/ congenital heart diseases Y / N / U

8. Vaccination: (Code***:*** **0**: none; **9**: unknown)

Type of vaccines	**Total doses** **0**: none; **9**: unknown	**Age of the last dose** **99**: unknown	**Notes**
H. influenzae type b			
N. meningitidis			
S. pneumoniae			
Measles			
Mumps			
Rubella			
Chickenpox			
Sabin			
JE vaccine			

9. HIV: Positive/ Negative/ Unknown

PIG EXPOSURE

10. In patient's family, is there anybody that has job related to pigs? Y / N / U

- Pig breeder ☐
- Butcher ☐
- Slaughterer ☐
- Meat processor ☐
- Meat transporter ☐
- Pork roaster ☐
- Waiter ☐
- Veterinary inspector ☐

11. Does the patient have any pigs at home? Y / N / U

12. Have his/her pigs had any diseases in the last 4 weeks? Y / N / U

13. If yes, describe:

- Fever ☐
- Arthritis ☐
- Neurological problems ☐
- Cough ☐
- Sudden death ☐
- Miscarriage ☐
- Unknown ☐
- Others: ……

14. Has the patient done any of the work below during the last 2 weeks?

- Bathe the pigs ☐
- Feed pigs ☐
- Clean up the piggery ☐
- Slaughter pigs ☐
- Prepare/handle pig blood ☐
- Prepare/handle raw pork ☐
- Contact with pig's organs ☐
- Cook pork ☐
- Not done ☐
- Unknown ☐
- Others: ……

15. Which dishes did patient take in the last 2 weeks (especially when drinking)?

- Pig's blood curds ☐
- Duck's blood curds ☐
- Undercooked pig's blood ☐
- Pig tonsils or tongue ☐
- Pig stomach or intestines ☐
- Pig uterus ☐
- Not eat ☐
- Unknown ☐

CLINICAL MANIFESTATIONS ON ADMISSION

16. Days of illness: …….days

17. Antimicrobial therapy before admission? Yes/No/Unknown

If yes, could you describe:

……

……

18 Vital signs:

- Pulse: /minute
- Temperature: ^{0}C
- Blood pressure: / mmHg
- Breath rate: /minute

19. Glasgow Coma Scale (if child ≥ 3 year-old): E M V =

Blantyre Coma Scale (if child < 3 year-old): E M V =

Glasgow Coma Scale		Blantyre Coma Scale	
Eye opening (E)		***Eye movement (E)***	
Spontaneous	4	Watches or follows	1
To speech	3	Fails to watch or follow	0
To pain	2		
None	1		
Motor response (M)		***Best motor response (M)***	
Obeys commands	6	Localizes painful stimulus	2
Localizes pain	5	Withdraws limb from painful stimulus	1
Withdrawal	4	No response	0
Flexor response	3		
Extension	2		
None	1		
Verbal response (V)		***Best verbal response (V)***	
Oriented	5	Cries appropriately with pain	2
Confused	4	Moan or abnormal cry with pain	1
Inappropriate	3	No vocal response to pain	0
Incomprehensible	2		
None	1		

20. Clinical manifestations:

- Fever Y / N / U
- Chills/rigors Y / N / U
- Headache Y / N / U
- Nausea/vomiting Y / N / U
- Diarrhoea Y / N / U
- Dizziness Y / N / U
- Anorexia Y / N / U
- Bulging fontanelle Y / N / U
- Lethargy Y / N / U
- Delirium Y / N / U
- Confusion Y / N / U
- Unconscious Y / N / U
- Seizures Y / N / U
- Gastrointestinal haemorrhage Y / N / U
- Other haemorrhages Y / N / U

  ……

  …….

- Acrocyanosis/ Necrosis Y / N / U
- Rash Y / N / U

  ……

  ……
- Jaundice Y / N / U
- Hepatomegaly Y / N / U
- Urine retention Y / N / U
- Meningeal signs Y / N / U
- Hemiplegia Y / N / U
- Paraplegia Y / N / U
- Cranial nerve palsy Y / N / U
- Herpes labial Y / N / U
- Arthritis Y / N / U

  ……

  ……
- Ear drum:

  L Normal/ Abnormal

  R Normal/ Abnormal
- Heart exam:

  Normal/ Abnormal

  ……

  ……
- Lung exam:

  Normal/ Abnormal

  ……

  ……

LABORATORY FINDINGS ON ADMISSION

21. CBC

WBC ...../µl (N.....%, L.....%, M.....%, E.....%)

Hb.....g/dl, Hct.....%

Plt...../ µl

22. Liver function tests:

SGOT .....U/l, SGPT.....U/l, Bilirubin $_{total}$ .....µmol/l

23. Renal function tests:

BUN .....mmol/l, Creatinine .....µmol/l

24. CSF:

Date				
Colour…				
Opening pressure				
Cell count + WC (N/L) + RC				
Protein				
Glucose (CSF/blood)				
Lactate (CSF/blood)				
Gram stain				
Culture				
PCR				
ELISA				

25. CSF culture and antibiogram:

……

……

……

……

26. Blood culture and antibiogram:

……

……

……

……

27. Chest X-ray: Normal / Abnormal / Not done

……

……

26. Cranial CT Scan/MRI: Normal / Abnormal / Not done

……

……

……

TREATMENT AND OUTCOME

27. Final diagnosis: .....

28. Dexamethasone: Y / N / U

29. Antimicrobial therapy:

……

……

……

……

30. Hearing status on discharge:

- Tinnitus Y / N / U
- Hearing loss Y / N / U
- Deaf (completely) Y / N / U

31. Other sequelae:

……

32. Outcome:

- Survival ☐
- Died ☐
- Transferred ☐
- Unknown ☐

31. Duration of staying in hospital: …… day(s).

II. Participant Information sheets and Consent forms:

a. Adult patient:

Hospital for Tropical Diseases
190 Ben Ham Tu, District 5
Ho Chi Minh City, Viet Nam

Hospital for Tropical Diseases: 08. 8380302

Contact physicians: Dr Ho Dang Trung Nghia: 0918500638

Patient information sheet

Oxtrec No.: 01 08

A study of causes of infection in southern and central Viet Nam

You are being invited to take part in a research study on the causes of Central Nervous System infections because you may have an infection of your Central Nervous System. Please read this information sheet carefully or have someone read it for you. You will be given a copy of this form to keep.

What is the reason for doing the study?

Central nervous system infection is a serious and life-threatening disease. The relative frequencies of the cause of meningitis/encephalitis have varied with periods of time, location, age, underlying medical and/or surgical conditions of patients. In Viet Nam, we have little information about why people get meningitis/encephalitis. We would like to get more information by studying patients with CNS infection. Your participation in this study does not have direct benefit for you now, but may have benefit for patients in the future. You can withdraw from this study at any time if you choose to.

What will happen if I take part in the study?

You will be given treatment for the disease, for which you have been admitted to the hospital, whether or not you decide to take part in the study.

What tests will be done?

A lumbar puncture will be done to collect some cerebrospinal fluid (CSF), this is fluid in your back, for meningitis/encephalitis diagnosis as part of standard clinical care. One millilitre of your CSF will be used to detect bacteria or viruses using molecular techniques. All samples will be labelled with a study number rather than your name, to protect your identity. Samples will be tested and stored indefinitely in a freezer at the Oxford University Clinical Research Unit, Hospital for Tropical Diseases. Further tests for detection of bacteria or viruses on stored samples, may be undertaken in the future to improve our understanding of the disease. We will not perform any analysis related to human genetics on your sample.

Risks

There are very few risks to you from being in the study. Collecting of the fluid from your back will hurt for a moment and may leave a bruise. This is part of your normal clinical care.

Confidentiality

Information about you will be kept confidential and will not be made available to anyone who is not connected with the study without your consent.

Cost

You will not have to pay for anything other than the normal cost of routine inpatient care. All tests related to the study will be paid for.

Questions

If you have any other questions about the study please contact:
Doctor's name: Dr Ho Dung Trung Nghia
Telephone number: 0918500638

CONSENT FORM

A study of causes of infection in southern and central Viet Nam

Oxtrec No. 01 08

Consent from patient

☐ I have read and understood the information sheet.

☐ I have been fully informed of the possible risks and benefits of taking part in this study, and agree to take part. I know that I can withdraw at any time if I choose to.

☐ I also agree to the indefinite storage of my cerebrospinal fluid for further tests to detect bacteria or viruses at a later date.

Name of patient: _____Signature: _____

Name of physician: _____Signature: _____

Date: _____

If the patient gives verbal consent to take part in the study, but is unable to sign, the physician can record the consent here:

Name of physician: _____Signature: _____

Date: _____

Consent from relative

☐ I have read and understood the information sheet.

☐ I have been fully informed of the possible risks and benefits of taking part in this study, and agree for my relative _____to take part.

☐ I also agree to the indefinite storage of my relative's cerebrospinal fluid for further tests related to the cause of infection at a later date.

Name of relative: _____Signature: _____

Relationship to patient: _____

Name of physician: _____Signature: _____

Date: _____

Consent from two independent physicians

If the patient is unconscious, two independent doctors approached to act as proxy for relatives should not be involved in the study and also that proper consent will be sought as and when the patients recovers consciousness.

Name of physician: _____Signature: _____

Date: _____

Name of physician: _____Signature: _____

Date: _____

b. Paediatric patient:

Hospital for Tropical Diseases
190 Ben Ham TU, District 5
Ho Chi Minh City, Viet Nam

Hospital for Tropical Diseases: 08. 8380302

Contact physicians: Dr Ho Dang Trung Nghia: 0918500638

Patient information sheet (children)

Oxtrec No.: 01 08

A study of causes of infection in southern and central Viet Nam

Your child is being invited to take part in a research study on the causes of Central Nervous System infection. Please read this information sheet carefully or have someone read it for you. You will be given a copy of this form to keep.

What is the reason for doing the study?

We are asking to enroll your child in a study to find out what caused her/his illness (meningitis or encephalitis). We want to find out what causes this disease in Viet Nam. Meningitis and encephalitis are common diseases in Viet Nam, and we often do not know the cause or how to treat the disease. We hope this work will help us understand the disease, to prevent it happening and we hope to plan the best treatment for this disease for future generations of people in Vietnam. Your participation in this study does not have direct benefit for you now, but may have benefit for patients in the future. You can withdraw from this study at any time if you choose to.

What will happen if my child takes part in the study?

Your child will be given treatment for the disease, for which he/she has been admitted to the hospital, whether or not you decide to enroll in the study.

What tests will be done?

A lumbar puncture will be done to collect some cerebrospinal fluid (CSF), this is fluid in the back, for meningitis/encephalitis diagnosis as part of standard clinical care. One millilitre of the CSF will be used to detect bacteria or viruses using molecular techniques. All samples will be labelled with a study number rather than your child's name, to protect her/his identity. Samples will be tested and stored indefinitely in a freezer at the Oxford University Clinical Research Unit, Hospital for Tropical Diseases. Further tests for detection of bacteria or viruses on stored samples, may be undertaken in the future to improve our understanding of the disease. We will not perform any analysis related to human genetics on the sample.

Risks

There are very few risks to your child from being in our study. Collecting the fluid from their back will hurt for a moment and may leave a bruise. This is part of normal clinical care

Confidentiality

Information about your child will be kept confidential and will not be made available to anyone who is not connected with the study without your consent.

Cost

You will not have to pay for anything other than the normal cost of routine inpatient care. All tests related to the study will be paid for.

Questions

If you have any other questions about the study please contact:
Doctor's name: Dr Ho Dung Trung Nghia
Telephone number: 0918500638

CONSENT FORM (children)

A study of causes of infection in southern and central Viet Nam

Oxtrec No. 01 08

Consent from parent/caretaker

☐ I have read and understood the information sheet.

☐ I have been fully informed of the possible risks and benefits of taking part in this study, and agree for my child _____ to take part.

☐ I also agree to the indefinite storage of my child's cerebrospinal fluid for further tests related to the cause of infection at a later date.

Name of parent/caretaker: _____Signature: _____

Relationship to patient:_____

Name of physician: _____Signature: _____

Date: _____

If the parent/caretaker gives verbal consent to take part in the study, but is unable to sign, the physician can record the consent here:

Name of physician: _____ Signature: _____

Date: _____

Consent from two independent physician

If the patient is unconscious, two independent doctors approached to act as proxy for relatives should not be involved in the study and also that proper consent will be sought as and when the patients recovers consciousness.

Name of physician: _____Signature: _____

Date: _____

Name of physician: _____Signature: _____

Date: _____

III. The ethical approvals:

a. HTD's approval:

Sở Y tế Tp. Hồ Chí Minh
BỆNH VIỆN BỆNH NHIỆT ĐỚI

CỘNG HÒA XÃ HỘI CHỦ NGHĨA VIỆT NAM
Độc lập – Tự do – Hạnh phúc

TP. Hồ Chí Minh, ngày 20 tháng 02 năm 2008

KẾT QUẢ XÉT DUYỆT ĐỀ CƯƠNG NGHIÊN CỨU KHOA HỌC
CỦA HỘI ĐỒNG KHOA HỌC CÔNG NGHỆ VÀ Y ĐỨC BVBNĐ

I. Thành phần tham dự:

Chủ tọa: PGS.TS. Nguyễn Trần Chính

Thư ký: ThS. Võ Minh Quang

Thành viên: TS. Trần Tịnh Hiền, ThS. Lâm Minh Yến, ThS. Nguyễn Văn Vĩnh Châu, TS. Đông Thị Hòai Tâm, TS. Lê Thị Thu Thảo, BS. Nguyễn Minh Dũng, BS. Nguyễn Ngọc Vinh, ThS. Đinh Nguyễn Huy Mẫn, ThS. Trần Phủ Mạnh Siêu

Thời gian: 9h30 – 11h30 ngày 24/01/2008 tại phòng giao ban bệnh viện

II. Nội dung xét duyệt:

Tên đề cương: Khảo sát các tác nhân gây bệnh thường gặp trong bệnh lý nhiễm trùng hệ thần kinh trung ương ở người tại miền Nam và miền Trung Việt Nam

Cấp quản lý: Cơ sở

Chủ nhiệm: TS. Trần Tịnh Hiền

Kết luận: **Thông qua**

Mã số nghiên cứu: **CS/NĐ/08/12**

Yêu cầu:
- Khi tiến hành nghiên cứu Chủ nhiệm đề tài phải tuân thủ chặt chẻ nội dung đề cương được duyệt, bất cứ thay đổi nào khác với đề cương đã duyệt đều phải có sự chấp thuận của HĐKHCN&YĐ trước khi thực hiện.
- Báo cáo tiến độ nghiên cứu định kỳ mỗi 3 tháng theo quy định

Chủ tịch HĐ
GIÁM ĐỐC

PGS.TS. NGUYỄN TRẦN CHÍNH

Thư ký

ThS. VÕ MINH QUANG

b. OXTREC's approval:

Oxford Tropical Research Ethics Committee
OXTREC
University of Oxford
Room 13, 1st Floor, Manor House
The John Radcliffe, Headington, Oxford OX3 9DZ
tel. +44 (0) 1865 743005, fax +44 (0) 1865 743 002
e-mail: Fiona.Maclean@admin.ox.ac.uk

26th February 2008

Dr Tran Tingh Hien
Hospital for Tropical Diseases
190 Ben Ham Tu
Ho Chi Minh City
Viet Nam

Dear Dr Hien

Full Title of Study: The Etiology of Central Nervous System Infections in Southern & Central Viet Nam

OXTREC Reference Number: 01 08

Thank you for the amendments and clarification for the above study received on 25th February 2008.

This completes our ethical review and I am happy to give OXTREC's approval. The study can commence once you have received local ethical approval.

Protocol V2.0 dated 25.02.08
PIS & Consent for Adult V2.0 Dated Feb 08
PIS & Consent for Child V2.0 Dated Feb 08

Yours sincerely

Richard Mayon-White.

Richard Mayon-White
OXTREC Chair

Appendix B

Case-control study to identify risk factors of *S. suis* infection in Viet Nam (EN study)

I. Case record forms:

a. Questionnaire:

Did you suffer from meningitis during the last year?

Study number: EN
Hospital number:
Name interviewer:
Date:
Time: until

QUESTIONNAIRE

1. GENERAL INFORMATION:

N^0	Question	Answer		
101	What's your name?	Initials /__/__/__/		
102	What's your address?	District Province Rural 1 Urban 2		
103	How long have you lived here?	<4 weeks ≥4 weeks	1 2	
N^0	**Question**	**Answer**	**Code**	**Other**
104	How old are you?	……years		Validated by ID card: yes/no
105	Sex	Male Female	1 2	
106	What is your principal occupation?	……. No job No answer	 98 99	
107	What is your secondary occupation?	……... No job No answer	 98 99	

108	What is your education level?	Primary Secondary “level 2” Secondary “level 3” University Illiterate Unknown No answer	1 2 3 4 5 98 99	
109	What is your religion?	Buddhism Catholicism Christianity Cao dai Other (specify)…….. No religion Unknown No answer	1 2 3 4 5 6 98 99	
110	What is your ethnic background?	Kinh Khmer Chinese Other (specify)…….. Unknown No answer	1 2 3 4 98 99	
111	Are you married?	Single Married Divorce/Separated No answer	1 2 3 99	
112	How many persons live in your house?	< 15 year-old…… ≥ 15 year-old…… no answer	 99	
113	In your family, is there anybody that has job related to pigs? (Pig breeder, butcher, abattoir worker, meat transporter, waiter, cook, pork roaster, meat processor, veterinary inspector, housewife…)	Yes No Unknown No answer If yes, specify…… ……	1 2 98 99	

114	How much do you earn a month (your income)?	VND……..……			
		Unknown	98		
		No answer	99		
115	Do you own …?		Yes	No	
		TV	1	2	Multiple
		Motorbike	1	2	answers
		Car	1	2	possible
		House category 1-3	1	2	
		House category 4	1	2	
		Thatched house	1	2	
		None of the above	3		
		No answer	99		

2. MEDICAL HISTORY:

201	Have you had any diseases? (except for current illness)	Yes	1	
		No	2	
		If yes, specify…… ……..		
		Unknown	98	
		No answer	99	
202	Have you ever been hospitalized? (except for current hospitalization)	Yes	1	If yes, for which diseases?
		No	2	
		Unknown	98	
		No answer	99	

203	Which diseases/conditions did you have or are you still suffering from?	Diseases/conditions		V*	Time frame*
		Nothing	0		
		Bacterial meningitis	1		
		Diabetes mellitus	2		
		Malignancy	3		
		Valvular heart diseases	4		
		Hydrocephalus	5		
		Splenectomy	6		
		Alcoholic	7		
		……			
		……			
V*: Validated by hospital records : yes(1)/no(2) for each illness Time frame*: Time frame for each illness: when and how long					
204	Are you using any medication (in the last 4 weeks, at least 2 days per week)?	Drugs	Yes	No	Validated by hospital records or direct inspection
		Steroids	1	2	Yes / No
		Antibiotics	1	2	Yes / No
		……	1	2	Yes / No
		……	1	2	Yes / No
		No treatment	0		
		Unknown	98		
		No answer	99		
205	Have you had any disease in the last 2 weeks?	Yes	1		
		No	2		
		Unknown	98		
		No answer	99		
206	If yes, could you describe diseases/ symptoms/ signs?	Common cold/Flu	1		
		Diarrhoea	2		
		Otorrhea/Otalgia	3		
		Extraction of your teeth	4		
		Other (specify)……..	5		
		……			

207	Have you had any skin injury (cut, burn…) in the last 2 weeks?	Yes No Unknown No answer	1 2 98 99	
208	If yes, describe your skin injuries? • Location (all) • Extend • Open/closed	…… …… …… …… …… …… …… ……		
209	Have you had any head injury? (in the last 2 weeks)	Yes No Unknown No answer	1 2 98 99	

3. CONTACT WITH PIGS:

301	Do you have any pigs at home, now or in the last 6 months?	Yes No Unknown No answer	1 2 98 99	If No, go to Q308
302	How many pigs do you have at home?	……pigs Unknown No answer	 98 99	
303	How long have you kept them at home?	……months Unknown No answer	 98 99	
304	Where do you keep your pigs?	In piggery Let wander Unknown No answer	1 2 98 99	

305	Why do you breed pigs?		Yes	No	
			1	2	
		Reproduction	1	2	
		Selling	1	2	
		Eating	98		
		Unknown	99		
		No answer			
306	Have your pigs had any diseases in the last 4 weeks?	Yes	1		
		No	2		
		Unknown	98		
		No answer	99		
307	If yes, describe?		Yes	No	
		Fever	1	2	
		Arthritis	1	2	
		Neurological problems	1	2	
		Cough	1	2	
		Sudden death	1	2	
		Miscarriage	1	2	
		Unknown	98		
		No answer	99		
308	Have you done any of the work below during the last 2 weeks?		Yes	No	Frequency (times/week),
		To bathe your pigs	1	2	
		To feed pigs	1	2	
		To clean up the piggery	1	2	
		slaughter pigs	1	2	
		prepare/handle pig blood	1	2	
		prepare/handle raw pork	1	2	
		cook pork	1	2	
		Unknown	98		
		No answer	99		

309	Do your neighbours have any pigs?	Yes No Unknown No answer	1 2 98 99		
310	Did you visit a pig farm or a house that has some pigs in the last 2 weeks?	Yes No Unknown No answer	1 2 98 99		
311	Did you have contact with pork blood or other pig's organs in the last 2 week?	Yes No Unknown No answer	1 2 98 99		
312	If yes, describe?	…….. …….. ……..			Frequency (times/week)
313	Other contact with pigs in the last 2 weeks?	Hunting wild boar Other (specify)…… …….. ……..	1 2		
314	Have you had any animals at home in the last 2 weeks?	Yes No Unknown No answer	1 2 98 99		
315	If yes, what kind of animal?		Yes	No	
		Dog	1	2	
		Cat	1	2	
		Cow	1	2	
		Chicken	1	2	
		Duck	1	2	
		Other……	1	2	
		Unknown	98		
		No answer	99		

4. EATING HABITS AND SANITARY

401	Do you eat pork?	Yes No Unknown No answer	1 2 98 99		
402	How many times do you eat pork each week?	……times Unknown No answer	 98 99		
403	Which dishes?	Dishes	Yes	No	Frequency (times/week)
		Duck/Pig's blood curds Fermented pork roll Pork meat Pig tonsils/tongue Pig stomach or intestines Pig uterus Meat of wild boar Other …….. Unknown No answer	1 1 1 1 1 1 1 1 98 99	2 2 2 2 2 2 2 2	
404	Do you drink beer/wine?	Yes No Unknown No answer	1 2 98 99		If No, go to question 408
405	If Yes, could you describe?	Frequency times/week	How much? (ml/each time)	How long? (years)	
	* Beer				
	* Wine				
		* Unknown: 98, No answer: 99			

406	Where do you usually drink beer/wine?	Where	Yes	No	
		At home	1	2	
		In the restaurant	1	2	
407	Which dishes do you usually take when drinking?	Dishes	Yes	No	
		Pork meat	1	2	
		Beef meat	1	2	
		Pig's blood curds	1	2	
		Duck's blood curds	1	2	
		Pig tonsils/tongue	1	2	
		Pig stomach	1	2	
		Pig uterus	1	2	
		Meat of wild boar	1	2	
		……			
		……			
		Unknown	98		
		No answer	99		
408	Do you usually smoke?	Yes	1		
		No	2		
		Unknown	98		
		No answer	99		
409	If yes, do you usually smoke when you *contact** with pig? (*contact**: To bathe your pigs, to feed pigs, to clean up the piggery, slaughter pigs, prepare/handle pig blood, prepare/handle raw pork, cook pork).	Yes	1		
		No	2		
		Unknown	98		
		No answer	99		
410	Do you wash your hands after going to the toilet?	Yes	1		
		No	2		
		Unknown	98		
		No answer	99		

411	Do you wash your hands before and after preparing meals?	Yes No Unknown No answer	1 2 98 99		
412	Which sources of water do you use in your house for drinking?	Sources of water	Yes	No	Multiple answers possible
		Tap-water Water from the well Rain water Water from the river Water from the pond/lake	1 1 1 1 1	2 2 2 2 2	
		Unknown No answer	98 99		
413	Does your house have a toilet?	Yes No Unknown No answer	1 2 98 99		
414	Where do you and your family defecate?		**Yes**	**No**	
		Toilet In the field In the river In the fish pond/lake	1 1 1 1	2 2 2 2	
		Unknown No answer	98 99		

This is the end of the interview. Thank you for your time answering these questions.

b. Data form:

DATA FORM

Study number: EN

Hospital number:

GENERAL INFORMATIONS

1. Name: ..... Age: .....
2. Sex: ❑ (Male= 1, Female= 2)
3. Address: ..... Commune: .....
   District: ..... Province: .....
   ❑ (Rural=1, Urban=2)
4. Occupation: .....
5. Ethnic groups: .....
6. Date of admission: .....
7. Reason of admission: .....

UNDERLYING DISEASES or PAST MEDICAL HISTORY

8. Did the patient have any underlying diseases or past medical history?
   (Yes=1, No=2, Unknown=99)

Diabetes	❑	Alcoholic	❑
Otitis	❑	Splenectomy	❑
Head trauma	❑	Pig exposure	❑
Heart diseases	❑		

9. Others, could you describe? .....
   .....
   .....

CLINICAL MANIFESTATIONS ON ADMISSION

10. Days of illness:.....days.

11. Antimicrobial therapy before admission? ❑

(Yes=1, No=2, Unknown=99)

If yes, could you describe?.....

.....

.....

12. Vital signs:

Date														
40^0C														
T														
36^0C														
Pulse														
BP														
RR														
Urine Output														
GCS														

13. GCS on admission: E M V =

Question 14 - 42, (Yes=1, No=2, Unknown=99)

14. Fever ❑

15. Chills, rigors: ❑

16. Headache: ❑

17. Nausea/Vomiting: ❑

18. Diarrhoea: ❑

19. Dizziness: ❑

20. Tinnitus: ❑

R/L/Both sides

21. Delirium: ❑

22. Confusion: ❑

23. Unconscious: ❑

24. Seizures: ❑ if yes, describe .....

25. Gastrointestinal haemorrhage: ❑

26. Other haemorrhages: ❑, if yes, describe .....

27. Acrocyanosis: ❑

28. Ischemic necrosis of peripheral tissues: ❑

29. Rash: ❑, if yes, describe rash including location: .....

.....

30. Skin injuries: ❑, if yes, describe lesions including location: .....

.....

CASE NO. _____ NAME _____

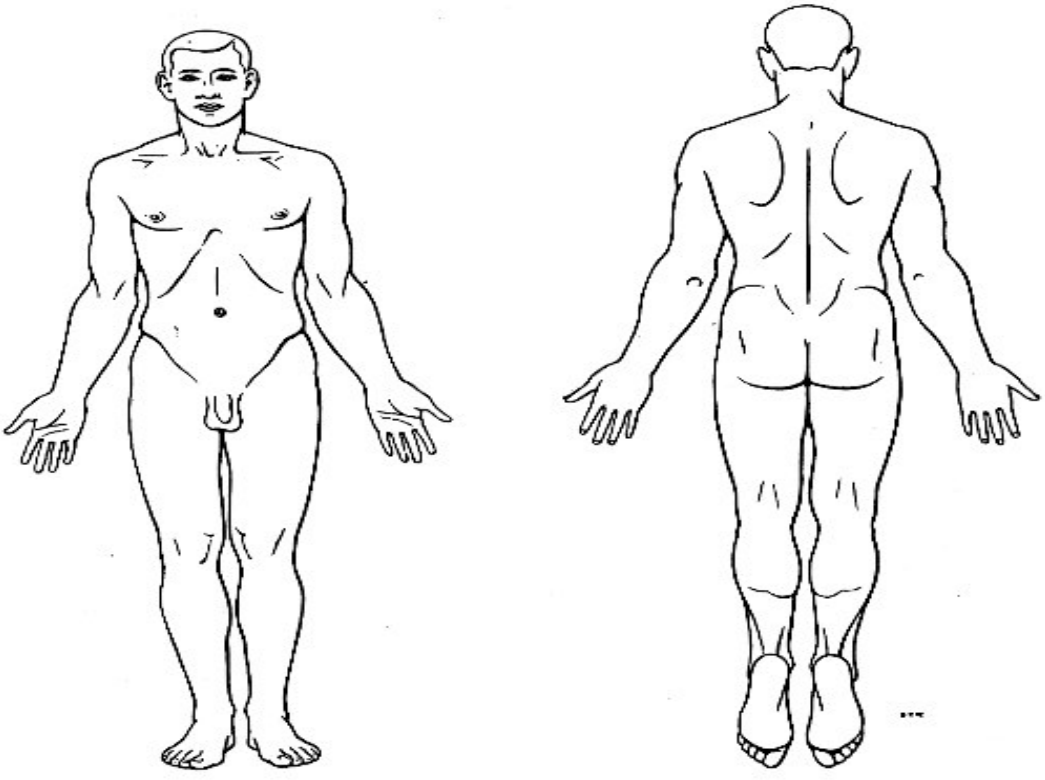


DIAGRAM A

Figure 25 (17)

31. Jaundice: ❑

32. Hepatomegaly: ❑

33. Urine retention: ❑

34. Neck stiffness: ❑

35. Kernig's sign: ❑

36. Brudzinski's sign: ❑

37. Focal neurological findings: ❑ if yes, describe.....

38. Abnormal cranial nerve: ❑ if yes, describe.....

39. Papilledema: ❑

40. Deafness: ❑ R/L/Both sides

41. Herpes labial: ❑

42. Arthritis: ❑, if yes, describe .....

43. Ear drum: (Normal=1, Abnormal=2)

R ❑ L ❑

44. Heart exam: ❑(Normal=1, Abnormal=2).....

45. Lung exam: ❑(Normal=1, Abnormal=2) .....

46. Teeth condition: .....

47. Other symptoms:.....

.....

.....

48. Complications: ❑

Septic shock = 1 DIC = 2 ARDS = 3 Renal failure = 4

None = 0

Other ……

LABORATORY FINDINGS ON ADMISSION

49. CBC

WBC ..... /µl (N ..... %, L ..... %, M ..... %, E .....%)

Hb……g/dl, Hct..... %

Plt ..... / µl

50. Liver function tests:

SGOT ..... U/l, SGPT ..... U/l, $Bilirubin_{total}$..... µmol/l

51. Renal function tests:

BUN ..... µmol/l, Creatinine…… µmol/l

52. DIC tests:

TT..... , PT ....., Fibrinogen ....., TCK.....

53. Blood gases:

pH..... $PaCO_2$..... PaO_2 ..... HCO^-_3 .....

Conclusion:

54. CSF:

Date				
Colour…				
OP				
Cell count + WC (N/L) + RC				
Protein				
Glucose (CSF/blood)				
Lactate (CSF/Blood)				
Gram stain/ AFB/ India ink				
Culture				
PCR				

55. CSF culture and antibiogram:

.....

.....

.....

.....

56. Blood culture and antibiogram:

.....

.....

.....

.....

57. Throat and rectal swabs (PCR):

Samples	Date	PCR results	Other
Throat swab I			
Throat swab II			
Rectal swab I			
Rectal swab II			

TREATMENT AND OUTCOME

58. Final diagnosis: .....

.....

59. Dexamethasone: ❑ (Yes=1, No=2, Unknown=99)

60. Antimicrobial therapy:

.....

.....

.....

61. Hemofiltration: ❑ (Yes=1, No=2, Unknown=99)

62. Mechanical ventilation: ❑ (Yes=1, No=2, Unknown=99)

63. Outcome: ❑ (Survival=1, Died=2, Transferred=3, Unknown=99)

64. Considered mentally normal by medical staff: ❑ (Normal=1, Personality difference=2, Moderately abnormal=3, Severely abnormal=4)

65. Understanding: ❑ (Same as before illness=1, Reduce=2, None=3)

66. Able to sit: ❑ (Independent=1, With help=2, Not at all = 3)

67. Able to stand: ❑ (Independent=1, With help=2, Not at all = 3)

68. Able to walk: ❑ (Independent=1, With help=2, Not at all = 3)

69. Power:

R arm: /5 L arm: /5

R leg: /5 L leg: /5

70. Cerebellar syndrome:

Gait: ❑ (Normal=1, Abnormal=2)

Finger nose test ❑ (Normal=1, Abnormal=2)

II. Participant Information sheets and Consent forms:

a. Patient:

Hospital for Tropical Diseases
190 Ben Ham TU, District 5
Ho Chi Minh City, Viet Nam

Hospital for Tropical Diseases: 8. 8380302
Contact physicians: Dr Ho Dung Trung Nghia: 0918500638
Dr Constance Schultsz: 8. 9237954

Patient information sheet

Oxtrec No.:012-06

A case-control study to identify risk-factors for *Streptococcus suis* meningitis and septicaemia in adults in southern Viet Nam

You are being invited to take part in a research study of *Streptococcus suis* infection. Please read this information sheet carefully or have someone read it for you. You will be given a copy of this form to keep.

What is the reason for doing the study?

Streptococcus suis meningitis is an important disease in Viet Nam. We know that pigs can carry *Streptococcus suis* in their body and that they can become ill due to infection with *Streptococcus suis*. At this time, we have little information on how humans get infected with *Streptococcus suis* and what measures we can take to prevent infection. We would like to get more information by studying patients with *Streptococcus suis* infection, patients with other infections, and healthy people, and compare the results. Your participation in this study does not have direct benefit for you now, but may have benefit for patients in the future. You can withdraw from this study at any time if you choose to.

What will happen if I take part in the study?

- You will be given treatment for the disease, for which you have been admitted to the hospital, whether or not you decide to take part in the study.
- You will be asked a number of questions, using a questionnaire. It takes you about 15 minutes. We will ask you these questions when you are fully conscious and capable of answering any questions.

What tests will be done?

- You will have 2 throat swabs taken to detect the presence or absence of *Streptococcus suis* in your throat. The first sample will be taken after giving consent to participate in the study, and the second 14 days after admission or at discharge.
- You will have 2 stool samples or rectal swabs, whichever you prefer, taken to detect the presence or absence of *Streptococcus suis* in your large intestine. The first sample will be taken after giving consent to participate in the study, and the second 14 days after admission or at discharge.
- You will be taken 10 ml blood
    - to detect if your body has produced antibodies against *Streptococcus suis*
    - to assess whether certain people are genetically susceptible to this disease.
- If you have pigs at home, we will take throat swab samples from some of the piglets or pigs to detect the presence of *Streptococcus suis* in your pigs.
- We will ask 3 of your adult household members to also participate in this study by supplying swab samples.

Taking a throat or rectal swab sample can be a little uncomfortable. Taking blood may cause a little pain and a bruise.
Taking a throat swab sample from a piglet or pig causes a little stress for the animal.

All samples will be labelled with a study number rather than your name, to protect your identity. Samples will be tested and stored indefinitely in a freezer at the Oxford University Clinical Research Unit, Hospital for Tropical Diseases. Further tests on stored samples, including studies on your DNA (your genetic make up), may be undertaken in the future to improve our understanding of the disease.

If we detect *Streptococcus suis* in any of the samples, we will inform you by mail. At the moment we think that this does not require any additional treatment.

Confidentiality

Information about you will be kept confidential and will not be made available to anyone who is not connected with the study without your consent.

Cost

You will not have to pay for anything other than the normal cost of routine inpatient care. All tests related to the study will be paid for.

Questions

If you have any other questions about the study please contact:
Doctor's name: Dr Ho Dung Trung Nghia
Telephone number: 0918500638

CONSENT FORM

A case-control study to identify risk-factors for *Streptococcus suis* meningitis and septicaemia in adults in southern Viet Nam

Oxtrec No. 012-06

Consent from patient

☐ I have read and understood the information sheet.

☐ I have been fully informed of the possible risks and benefits of taking part in this study, and agree to take part. I know that I can withdraw at any time if I choose to.

☐ I also agree to the indefinite storage of my swab samples and blood for further tests, including genetic tests, related to *Streptococcus suis* infection, at a later date.

☐ I also agree to have throat swab samples taken from some of the pigs in my household to detect the presence of *Streptococcus suis*.

Name of patient: _____Signature: _____

Name of physician: _____ Signature: _____

Date: _____

If the patient gives verbal consent to take part in the study, but is unable to sign, the physician can record the consent here:

Name of physician: _____ Signature: _____

Date: _____

Consent from relative

☐ I have read and understood the information sheet.

☐ I have been fully informed of the possible risks and benefits of taking part in this study, and agree for my relative _____to take part.

☐ I also agree to the indefinite storage of my relative's swab samples and blood for further tests related to *Streptococcus suis* infection at a later date.

☐ I also agree to have throat swab samples taken from some of the pigs in my relative's household to detect the presence of *Streptococcus suis*.

Name of relative: _____ Signature: _____
Relationship to patient: _____
Name of physician: _____ Signature: _____
Date: _____

Consent from two independent physicians

If the patient is unconscious, two independent doctors may decide to enter the patient into the study.

Name of physician: _____ Signature: _____
Date: _____

Name of physician: _____ Signature: _____
Date: _____

b. Healthy control:

Hospital for Tropical Diseases
190 Ben Ham TU, District 5
Ho Chi Minh City, Viet Nam

Hospital for Tropical Diseases: 8. 8380302
Contact physicians: Dr Ho Dung Trung Nghia: 0918500638
Dr Constance Schultsz: 8. 9237954

Healthy control information sheet

Oxtrec No.:012-06

A case-control study to identify risk-factors for *Streptococcus suis* meningitis and septicaemia in adults in southern Viet Nam

You are being invited to take part in a research study of *Streptococcus suis* infection. Please read this information sheet carefully or have someone read it for you. You will be given a copy of this form to keep.

What is the reason for doing the study?

Streptococcus suis meningitis is an important disease in Viet Nam. We know that pigs can carry *Streptococcus suis* in their body and that they can become ill due to infection with *Streptococcus suis*. At this time, we have little information on how humans get infected with *Streptococcus suis* and what measures we can take to prevent infection. We would like to get more information by studying patients with *Streptococcus suis* infection, patients with other infections, and healthy people, and compare the results. We would like to invite you to be one of the healthy people participating in this study. Your participation in this study does not have direct benefit for you now, but may have benefit for patients in the future. You can withdraw from this study at any time if you choose to.

What will happen if I take part in the study?

- You will be asked a number of questions, using a questionnaire. It takes you about 15 minutes.

What tests will be done?

- You will have 2 throat swabs taken to detect the presence or absence of *Streptococcus suis* in your throat. The first sample will be taken after giving consent to participate in the study, and the second 14 days later.
- You will have 2 stool samples or rectal swabs, whichever you prefer, taken to detect the presence or absence of *Streptococcus suis* in your large intestine. The first sample will be taken after giving consent to participate in the study, and the second 14 days later.
- You will be taken 10 ml blood
    - to detect if your body has produced antibodies against *Streptococcus suis*
    - to assess whether certain people are genetically susceptible to this disease.
- If you have pigs at home, we will take throat swab samples from some of the piglets to detect the presence of *Streptococcus suis* in your pigs.
- We will ask 3 of your adult household members to also participate in this study by supplying swab samples.

Taking a throat or rectal swab sample can be a little uncomfortable. Taking blood may cause a little pain and a bruise.

Taking a throat swab sample from a piglet or pig causes a little stress for the animal.

All samples will be labelled with a study number rather than your name, to protect your identity. Samples will be tested and stored indefinitely in a freezer at the Oxford University Clinical Research Unit, Hospital for Tropical Diseases. Further tests on

stored samples, including studies of your DNA (your genetic make up), may be undertaken in the future to improve our understanding of the disease.

If we detect *Streptococcus suis* in any of the samples, we will inform you by mail. At the moment we think that this does not require any treatment.

Confidentiality

Information about you will be kept confidential and will not be made available to anyone who is not connected with the study without your consent.

Questions

If you have any other questions about the study please contact:

Doctor's name: Dr Ho Dung Trung Nghia

Telephone number: 0918500638

CONSENT FORM

A case-control study to identify risk-factors for *Streptococcus suis* meningitis and septicaemia in adults in southern Viet Nam

Oxtrec No. 012-06

Consent from healthy control

☐ I have read and understood the information sheet.

☐ I have been fully informed of the possible risks and benefits of taking part in this study, and agree to take part. I know that I can withdraw at any time if I choose to.

☐ I also agree to the indefinite storage of my swab samples and blood, for further tests, including genetic tests, related to *Streptococcus suis* infection, at a later date.

☐ I also agree to have throat swab samples taken from some of the pigs in my household to detect the presence of *Streptoccus suis*.

Name of control: _____ Signature: _____

Name of physician: _____ Signature: _____

Date: _____

If the control gives verbal consent to take part in the study, but is unable to sign, the physician can record the consent here:

As the investigator I confirm that the participant named below has been given the Information Sheet and has verbally agreed to participate in the study.

Name of control: _____

Name of physician: _____ Signature: _____

Date: _____

c. Household member:

Hospital for Tropical Diseases
190 Ben Ham TU, District 5
Ho Chi Minh City, Viet Nam

Hospital for Tropical Diseases: 8. 8380302
Contact physicians: Dr Ho Dung Trung Nghia: 0918500638
Dr Constance Schultsz: 8. 9237954

Information sheet
(household members)
Oxtrec No.:012-06

A case-control study to identify risk-factors for *Streptococcus suis* meningitis and septicaemia in adults in southern Viet Nam

You are being invited to take part in a research study of *Streptococcus suis* infection. Please read this information sheet carefully or have someone read it for you. You will be given a copy of this form to keep.

What is the reason for doing the study?

Streptococcus suis meningitis is an important disease in Viet Nam. We know that pigs can carry *Streptococcus suis* in their body and that they can become ill due to infection with *Streptococcus suis*. At this time, we have little information on how humans get infected with *Streptococcus suis* and what measures we can take to prevent infection. We would like to get more information by studying patients with *Streptococcus suis* infection, patients with other infections, and healthy people, and compare the results. We would also like to know if healthy individuals carry *S. suis* without any symptoms and if the presence of such carriers in the household increases the risk of infection. We would like to invite you to be one of those healthy household members participating in this study. Your participation in this study does not have direct benefit for you now, but may have benefit for patients in the future. You can withdraw from this study at any time if you choose to.

What will happen if I take part in the study?

- You will have 2 throat swabs taken, the first after giving consent during our first visit and the second 14 days later, to detect the presence of *Streptococcus suis* in your throat.
- You will have 2 stool samples or rectal swabs taken, whichever you prefer, the first after giving consent during our first visit and the second 14 days later, to detect the presence of *Streptococcus suis* in your large intestine.

Taking a throat or rectal swab sample can be a little uncomfortable.
All samples will be labelled with a study number rather than your name, to protect your identity. Samples will be tested and stored indefinitely in a freezer at the Oxford University Clinical Research Unit, Hospital for Tropical Diseases.

Further tests on stored samples may be undertaken in the future to improve our understanding of the disease.

If we detect *Streptococcus suis* in any of the samples, we will inform you by mail. At the moment we think that this does not require any treatment.

Confidentiality
Information about you will be kept confidential and will not be made available to anyone who is not connected with the study without your consent.

Questions
If you have any other questions about the study please contact:
Doctor's name: Dr Ho Dung Trung Nghia
Telephone number: 0918500638

CONSENT FORM

A case-control study to identify risk-factors for *Streptococcus suis* meningitis and septicemia in adults in southern Viet Nam

Oxtrec No. 012-06

Consent from household member

☐ I have read and understood the information sheet.

☐ I have been fully informed of the possible risks and benefits of taking part in this study, and agree to take part. I know that I can withdraw at any time if I choose to.

☐ I also agree to the indefinite storage of my swab samples for further tests related to *Streptococcus suis* infection at a later date.

Name of household member: _____ Signature: _____
Name of physician: _____ Signature: _____
Date: _____

If the household member gives verbal consent to take part in the study, but is unable to sign, the physician can record the consent here.

As the investigator I confirm that the participant named below has been given the Information Sheet and has verbally agreed to participate in the study.

Name of household member: _____
Name of physician: _____ Signature: _____
Date: _____

III. Ethical approvals:

a. HTD's approval:

Sở Y Tế Tp. Hồ Chí Minh
BỆNH VIỆN BỆNH NHIỆT ĐỚI
190 Bến Hàm Tử Q5

CỘNG HÒA XÃ HỘI CHỦ NGHĨA VIỆT NAM
Độc Lập - Tự Do - Hạnh Phúc

Thông Báo của Hội Đồng Khoa Học Kỹ Thuật và Y Đức
Bệnh Viện Bệnh Nhiệt Đới

Hội Đồng Khoa Học Công nghệ và Y Đức Bệnh Viện Bệnh Nhiệt Đới trong phiên họp ngày 26/1/2006 đã xem xét đề cương của đề tài

Xác định yếu tố nguy cơ của bệnh viêm màng não mủ và nhiễm trùng huyết do *Streptococcus suis* ở người lớn tại Việt Nam

Hội Đồng đã chấp thuận đề cương trên. Sự chấp thuận này có hiệu lực trong thời gian 04 năm kể từ ngày 26/01/2006. Những thay đổi trong đề cương, nếu có, đều phải được xem xét lại

Các thành viên của Hội Đồng tham dự duyệt xét:

- Nguyễn Trần Chính, MD, Ph.D	Phó Giám Đốc, Trưởng khoa Nhiễm B, Chủ nhiệm Bộ Môn Nhiễm, Khoa Y Đại Học Y Dược TP.HCM
- Trần Tịnh Hiền, MD, PhD, FRCP	Phó Giám Đốc, Trưởng khoa Nhiễm D
- Lê Thanh Chiến, MD	Phó Giám Đốc
- Nguyễn Minh Dũng, MD	Trưởng khoa HSCC Nhi
- Nguyễn Văn Vĩnh Châu, MD, MSc	Trưởng khoa Xét nghiệm
- Trần Phủ Mạnh Siêu, MD, MSc	Trưởng Labo Ký Sinh
- Lê thị Thu Thảo, MD, PhD	Trưởng khoa HSCC người lớn
- Nguyễn Ngọc Vinh, MD	Trương khoa Nội A
- Võ Minh Quang, MD, MSc	Phòng Kế hoạch tổng hợp

TP.HCM, ngày 14 tháng 2 năm 2006,
Chủ tịch Hội Đồng

SỞ Y TẾ THÀNH PHỐ HỒ CHÍ MINH
BỆNH VIỆN BỆNH NHIỆT ĐỚI

GIÁM ĐỐC

BS. Vương Hùng Việt

b. OXTREC's approval:

Oxford Tropical Research Ethics Committee
OXTREC
University of Oxford
Centre for Clinical Vaccinology and Tropical Medicine
Churchill Hospital, Old Road, Headington, OX3 7LJ
Telephone: +44 (0) 1865 857430 Fax: +44 (0) 1865 857 407
E-mail: oxtrec@ndm.ox.ac.uk

17 May 2006

Dr Constance Schultsz
The Hospital for Tropical Diseases
190 Ben Ham Tu
Quan 5
Ho Chi Minh City
Viet Nam

Dear Dr Schultsz

012-06: A case-control study to identify risk-factors for *Streptococcus suis* meningitis and septicaemia in adults in southern Viet Nam

Thank you very much for your letter about this study. The information sheet and consent forms are better now. Thank you for clarifying the veterinary aspects of the study.

We are happy to give approval. As you know, the study will be approved for three years in the first instance subject to a satisfactory annual report from you.

Yours sincerely

Dr Chris Conlon
Chairman

Appendix C

Publications arising from directly or linked with this thesis

Nghia, H. D., T. P. Le, M. Wolbers, Q. T. Cao, V. M. Nguyen, V. T. Tran, T. P. Le, H. P. Nguyen, T. H. Tran, X. S. Dinh, S. D. To, T. T. Hoang, T. Hoang, J. Campbell, V. V. Nguyen, T. C. Nguyen, V. D. Nguyen, T. H. Ngo, B. G. Spratt, T. H. Tran, J. Farrar and C. Schultsz (2011). **"Risk Factors of Streptococcus suis Infection in Vietnam. A Case-Control Study."** PLoS One **6**(3): e17604.

Hoa, N. T., T. T. Chieu, **H. D. Nghia**, N. T. Mai, P. H. Anh, M. Wolbers, S. Baker, J. I. Campbell, N. V. Chau, T. T. Hien, J. Farrar and C. Schultsz (2011). **"The antimicrobial resistance patterns and associated determinants in Streptococcus suis isolated from humans in southern Vietnam, 1997-2008."** BMC Infect Dis **11**: 6.

Nga, T. V. T, **H. D. Nghia**, L. T. P. Tu, T. S. Diep, N. T. Mai and C. Schultsz (2011). **"Real-time PCR for detection of Streptococcus suis serotype 2 in cerebrospinal fluid of human patients with meningitis."** Diagn Microbiol Infect Dis. **70**(4): 461-7.

Wertheim, H. F., **H. D. Nghia**, W. Taylor and C. Schultsz (2009). **"Streptococcus suis: an emerging human pathogen."** Clin Infect Dis **48**(5): 617-25.

Mai, N. T., N. T. Hoa, T. V. Nga, D. Linh le, T. T. Chau, D. X. Sinh, N. H. Phu, L. V. Chuong, T. S. Diep, J. Campbell, **H. D. Nghia**, T. N. Minh, N. V. Chau, M. D. de

Jong, N. T. Chinh, T. T. Hien, J. Farrar and C. Schultsz (2008). **"Streptococcus suis meningitis in adults in Vietnam."** Clin Infect Dis **46**(5): 659-67.

Nghia, H. D., N. T. Hoa, D. Linh le, J. Campbell, T. S. Diep, N. V. Chau, N. T. Mai, T. T. Hien, B. Spratt, J. Farrar and C. Schultsz (2008). **"Human case of Streptococcus suis serotype 16 infection."** Emerg Infect Dis **14**(1): 155-7.

Nguyen, T. H., T. H. Tran, G. Thwaites, V. C. Ly, X. S. Dinh, **T. N. Ho Dang**, Q. T. Dang, D. P. Nguyen, H. P. Nguyen, S. D. To, V. C. Nguyen, M. D. Nguyen, J. Campbell, C. Schultsz, C. Parry, M. E. Torok, N. White, T. C. Nguyen, T. H. Tran, K. Stepniewska and J. J. Farrar (2007). **"Dexamethasone in Vietnamese adolescents and adults with bacterial meningitis."** N Engl J Med **357**(24): 2431-40.

Torok, M. E., **H. D. Nghia**, T. T. Chau, N. T. Mai, G. E. Thwaites, K. Stepniewska and J. J. Farrar (2007). **"Validation of a diagnostic algorithm for adult tuberculous meningitis."** Am J Trop Med Hyg **77**(3): 555-9.

Chau, T. T., N. H. Mai, N. H. Phu, **H. D. Nghia**, L. V. Chuong, D. X. Sinh, V. A. Duong, P. T. Diep, J. I. Campbell, S. Baker, T. T. Hien, D. G. Lalloo, J. J. Farrar and J. N. Day (2010). **"A prospective descriptive study of cryptococcal meningitis in HIV uninfected patients in Vietnam - high prevalence of Cryptococcus neoformans var grubii in the absence of underlying disease."** BMC Infect Dis **10**: 199.

OPEN ACCESS Freely available online

PLoS one

Risk Factors of *Streptococcus suis* Infection in Vietnam. A Case-Control Study

Dang Trung Nghia Ho[1,2,3], Thi Phuong Tu Le[1], Marcel Wolbers[1], Quang Thai Cao[1], Van Minh Hoang Nguyen[1], Vu Thieu Nga Tran[1], Thi Phuong Thao Le[1], Hoan Phu Nguyen[3], Thi Hong Chau Tran[3], Xuan Sinh Dinh[3], Song Diep To[3], Thi Thanh Hang Hoang[1,2], Truong Hoang[2], James Campbell[1], Van Vinh Chau Nguyen[3], Tran Chinh Nguyen[3], Van Dung Nguyen[4], Thi Hoa Ngo[1], Brian G. Spratt[6], Tinh Hien Tran[1,3], Jeremy Farrar[1], Constance Schultsz[1,5]*

1 Oxford University Clinical Research Unit, Wellcome Trust Major Overseas Programme, Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam, **2** Pham Ngoc Thach University of Medicine, Ho Chi Minh City, Vietnam, **3** Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam, **4** Sub-Department of Animal Health Ho Chi Minh City, Ho Chi Minh City, Vietnam, **5** Center for Poverty-related Communicable Diseases, Academic Medical Centre, Amsterdam Institute of Global Health and Development, University of Amsterdam, Amsterdam, The Netherlands, **6** Department of Infectious Disease Epidemiology, Imperial College London, London, United Kingdom

Abstract

Background: *Streptococcus suis* infection, an emerging zoonosis, is an increasing public health problem across South East Asia and the most common cause of acute bacterial meningitis in adults in Vietnam. Little is known of the risk factors underlying the disease.

Methods and Findings: A case-control study with appropriate hospital and matched community controls for each patient was conducted between May 2006 and June 2009. Potential risk factors were assessed using a standardized questionnaire and investigation of throat and rectal *S. suis* carriage in cases, controls and their pigs, using real-time PCR and culture of swab samples. We recruited 101 cases of *S. suis* meningitis, 303 hospital controls and 300 community controls. By multivariate analysis, risk factors identified for *S. suis* infection as compared to either control group included eating "high risk" dishes, including such dishes as undercooked pig blood and pig intestine ($OR_1 = 2.22$; 95%CI = [1.15–4.28] and $OR_2 = 4.44$; 95%CI = [2.15–9.15]), occupations related to pigs ($OR_1 = 3.84$; 95%CI = [1.32–11.11] and $OR_2 = 5.52$; 95%CI = [1.49–20.39]), and exposures to pigs or pork in the presence of skin injuries ($OR_1 = 7.48$; 95%CI = [1.97–28.44] and $OR_2 = 15.96$; 95%CI = [2.97–85.72]). *S. suis* specific DNA was detected in rectal and throat swabs of 6 patients and was cultured from 2 rectal samples, but was not detected in such samples of 1522 healthy individuals or patients without *S. suis* infection.

Conclusions: This case control study, the largest prospective epidemiological assessment of this disease, has identified the most important risk factors associated with *S. suis* bacterial meningitis to be eating 'high risk' dishes popular in parts of Asia, occupational exposure to pigs and pig products, and preparation of pork in the presence of skin lesions. These risk factors can be addressed in public health campaigns aimed at preventing *S. suis* infection.

Citation: Ho DTN, Le TPT, Wolbers M, Cao QT, Nguyen VMH, et al. (2011) Risk Factors of *Streptococcus suis* Infection in Vietnam. A Case-Control Study. PLoS ONE 6(3): e17604. doi:10.1371/journal.pone.0017604

Editor: Tara Smith, University of Iowa, United States of America

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Competing Interests: The authors have declared that no competing interests exist.

* E-mail: schultsz@gmail.com

Introduction

The importance of zoonotic emerging infections is increasingly recognized, as illustrated by outbreaks of SARS coronavirus and the ongoing threat of human infections with avian influenza H5N1 virus. It is essential to understand transmission dynamics and risk factors for infection, in order to design effective strategies to contain and prevent the spread of zoonotic diseases [1]. *Streptococcus suis* infection is an emerging zoonotic infectious disease, which is increasingly reported in Asia. It has become an important threat for human health, illustrated by the explosive outbreak in Sichuan Province China associated with at least 215 cases and 39 deaths in 2005 [2]. In Vietnam, *S. suis* infection was first reported in 1996 and the number of human cases has increased annually. In Ho Chi Minh City and Hanoi, it causes approximately 40% of all adult acute bacterial meningitis cases. This is more than *Streptococcus pneumoniae* and *Neisseria meningitidis* combined [3,4,5].

S. suis is a Gram-positive, facultatively anaerobic coccus, which can be a commensal or pathogen for a wide range of mammalian species, particularly pigs. The natural habitat of *S. suis* in pigs is the upper respiratory-, the genital- and alimentary tracts. Based on differences in antigenic properties of the polysaccharide capsule, 33 serotypes have been distinguished to date, among which serotype 2 is most commonly associated with invasive disease in both pigs and humans. To date little is known of the risk factors underlying the disease or the portals of entry in humans. From small case series, the reported risk factor included occupational

exposure, such as in slaughter house workers, butchers, and pig breeders, meat processing, and pig transport. It is hypothesized that patients may be infected through minor cuts or abrasions on their skin [2,6]. However, whilst occupational exposure to pigs or pork was documented in 88% of the European patients described, it was reported in less than 50% of Asian cases [4,7], suggesting the contribution of other behavioural or exposure related risk factors in Asian populations, such as culinary habits or close proximity of pigs within households. In addition, whilst it is known that pigs can carry *S. suis* asymptomatically, it is not known if there is asymptomatic carriage of *S. suis* in humans, which could potentially contribute to an increased risk of infection, and to the possibilitiy of person-to-person transmission. We conducted a prospective case-control study to identify the risk factors of *S. suis* infection in Viet Nam.

Methods

Ethical approval

The study was approved by the Scientific and Ethics Committee of the Hospital for Tropical diseases and the University of Oxford Tropical Research Ethics Committee (OXTREC 012-06).

Study design and setting

This study was designed as a case-control study, including patients with invasive *S. suis* infection, an unmatched hospital control group and a community control group matched by residency and age, at a ratio of 1:3. The study was conducted at the Hospital for Tropical Diseases (HTD), a tertiary referral hospital of infectious diseases in the south of Viet Nam, between May 2006 and June 2009. The recruitment of cases and hospital control groups took place at the dedicated Central Nervous System (CNS) infectious disease ward at HTD. Community controls were recruited according to the residency of the cases in the south of Viet Nam, mainly in Ho Chi Minh City and the provinces of the Mekong River Delta.

Participants

Consecutive patients admitted with signs and symptoms consistent with central nervous system (CNS) infection were eligible for the study (Table 1). When *S. suis* infection was confirmed, patients were included as cases. After inclusion of a patient as a case, the next three consecutive patients admitted to the ward who met the inclusion criteria were included as hospital controls. Three community controls, matched for age (within a 10 year age range), were randomly identified from a list of eligible households available at the health center in the community of residence of the case, by using random number tables (Figure 1). Written informed consent was obtained from all patients and controls or their care takers.

Assessment of risk factors

Risk factors were assessed using a standardized questionnaire. This questionnaire was developed in Vietnamese and validated at HTD and consisted of four parts; socio-demographic and cultural factors, medical history, potential exposure to pigs or pork and culinary habits and hygiene measures. We hypothesized that consumption of "high risk" food dishes (Table 2), potentially contaminated with *S. suis*, could function as a source of infection. The majority of the questions were "closed questions" but "open questions" which allowed participants to explain in their own words were also included. Patients and hospital controls were interviewed when they were fully conscious. The questionnaire was filled in by structured interview, which was carried out by one of two research nurses on the ward for cases and hospital controls, or at the residency of community controls. The interviewers were blinded towards the diagnosis of the patient in the case and hospital control groups.

Collection of swabs for detection of *S. suis* serotype 2 carriage

From all patients and controls, throat and rectal swab samples were taken on admission or at the community visit and a second set of swab samples was taken ten to fourteen days later. As detection of potential carriage in cases and hospital controls could be affected by their antimicrobial treatment, household members were also studied for carriage of *S. suis* serotype 2 since if carriage and associated transmission were to occur, household members of carriers are the most likely to become positive. Adult household members, defined as any adult (at least 15 years old) who resided for at least 50% of the

Table 1. Inclusion and exclusion criteria.

Inclusion criteria	Exclusion criteria
Cases:	Did not provide informed consent.
At least 15 year-old	Recent history of bacterial meningitis (<1 year before admission).
Streptococcus suis meningitis or sepsis confirmed by blood culture, CSF culture, or CSF real-time PCR	Did not regain full consciousness (GCS 15) within 14 days after admission.
Admitted to CNS disease ward	Transferred to other hospitals within 7 days after admission
Hospital controls:	HIV positive.
At least 15 year-old	
Confirmed bacterial meningitis (not *S. suis*), eosinophilic meningitis, cryptococcal meningitis, viral encephalitis/meningitis* or malaria (confirmed by blood smear)	
Admitted to CNS disease ward	
Community controls:	
Living in the same commune as case for at least 4 weeks until inclusion of case	
Age matched with case (10 years range)	

(*) Viral encephalitis/meningitis was diagnosed on the basis of confirmation by positive diagnostic PCR or serology of CSF sample, or if the patient completely regained consciousness during treatment with antimicrobial agents for a duration of 48 hours or less.

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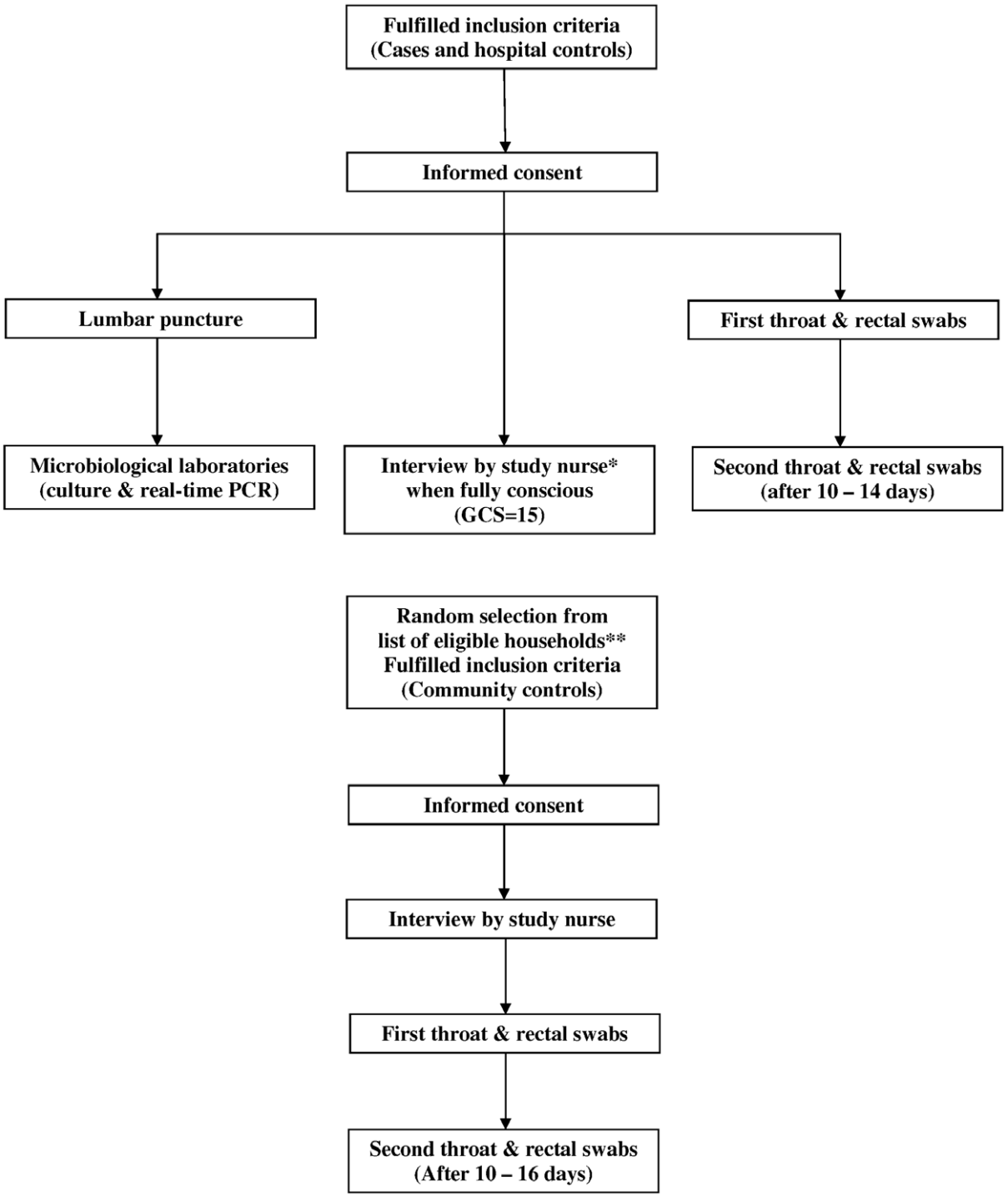

Figure 1. Flow diagram of recruitment of cases and controls. *Study nurses were unaware of case or control status of patients. ** Eligible households were defined as households in the same commune as a case.
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week in the same house as the case or control, were identified either at the HTD, when taking care of a case or hospital control, or during the household visits. From each household, we choose a maximum of three adult household members from whom we took throat and rectal swabs in the same way as for the cases and controls, following written informed consent.

For all cases and controls who reported exposure to pigs at home, sampling of the pigs was performed in collaboration with the Sub-department of Animal Health of Ho Chi Minh City. Swab samples were taken from all pigs present (except pregnant sows to avoid stress-induced miscarriage) within 4 weeks of admission of the patient, or at the second visit to the community controls.

Microbiological investigations

Culture of blood and cerebrospinal fluid was performed in the microbiology laboratory of the HTD using standard culture

Table 2. Definitions.

Occupational exposures: *at least one of the following occupations*
Butcher
Pig breeder
Slaughterer
Meat transporter
Meat processing
Veterinarian
Cook
Contact with pigs/pork: *at least one of the following contacts*
Bathe pigs
Feed pigs
Clean up the piggery
Slaughter pigs
Prepare or handle blood, organs from pigs
Visit a pig farm in the last 2 weeks
''High risk'' dishes:
Pig/duck fresh blood
Pig tonsils/tongue
Pig stomach/intestines
Pigs uterus
Under-cooked pig blood
Skin injuries:
Patients were checked by nurses and doctors for skin injuries on forearms, hands and feet. Injuries were defined as lesions with signs of disruption of skin integrity.
Underlying diseases:
alcoholism, diabetes mellitus and splenectomy.
Alcoholism:
A person drinking beer >1500 ml/day or wine >250 ml/day in at least 5 days/week (Wine = SPIRIT 30–40°)
Household exposure to pigs:
breeding any number of pigs at home.
Confirmed carriage:
A person with 2 PCR positive swab samples on two separate occasions, at least 10–14 days in between
Possible carriage:
A person with 1 PCR positive swab sample

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methods. Blood samples were taken on admission for all patients and blood cultures were performed using the BD BACTEC® 9050 blood culture system. *S. suis* was identified on the basis of colony morphology, negative catalase reaction, optochin resistance, and by APIStrep (Biomerieux, France) and subsequently serotyped (Statens Serum Institute, Denmark).

Swab samples were inoculated in transport medium (TRANS-WABS®, UK) at the site, and transferred to the laboratory at HTD or stored at 4°C until transfer within 48 hours. Samples were inoculated into selective Todd-Hewitt broth (OXOID, UK), containing Streptococcal Selective Reagent (Oxoid) and crystal violet [8] and incubated overnight at 37°C, followed by real-time PCR for detection of *S. suis* serotype 2. Positive samples were cultured to retrieve *S. suis* isolates.

We used an internally controlled real-time PCR for detection of *S. pneumoniae*, *H. influenzae* type b, *N. meningitidis* and *S. suis* serotype 2 in CSF samples [4,8,9,10]. The real-time PCR for detection of *S. suis* serotype 2 was also used on swab cultures. Previous validation of this PCR demonstrated a detection limit of 1–5 colony forming units per reaction [10].

After bacterial lysis, DNA was extracted using the EasyMag extraction system (BioMerieux, Ho Chi Minh City, Vietnam), according to manufacturer's instructions and subjected to consecutive monoplex PCR reactions (CSF samples).

Sample size

Demographic data obtained during a randomized study on the efficacy of adjunct dexamethasone for the treatment of acute bacterial meningitis showed that 59/226 (26%) of the non-*S. suis* patients had potential occupational exposure to pigs compared to 46/78 (59%) of *S. suis* meningitis patients [3], corresponding to an odds ratio of 4. We decided on a target sample size of 100 cases (and 300 matched controls), corresponding to a recruitment period of approximately 4 years and a target odds ratio for 80% power of 2.1 assuming a probability of exposure in controls of 0.25 and a correlation coefficient for exposure between matched cases and controls of at most 0.2. With 100 cases, we also expected to fit reliable multivariate models with up to 10 covariates without over fitting the data [11].

Statistical methods

All variables of interest were summarized by group (case, hospital, or community control). Categorical variables were summarized as number and percent (%). Continuous variables were summarized as median and interquartile range (IQR). To assess univariate associations of *S. suis* with potential risk factors in hospital controls, we used both logistic regression without any adjustment for covariates and with adjustment for sex, age, and living in a rural or urban area. We used conditional logistic regression for the matched community controls and these analyses were performed with and without additional adjustment for sex (in addition to the matched variables age and place of living). In a multivariate analysis of potential risk factors of main interest, all potential risk factors plus the potential confounders (e.g. age, sex, place of living) were jointly included in a logistic (hospital controls) or conditional logistic (community controls) regression model. No model selection such as backwards elimination was performed. We included separate effects of exposure to pigs or pork depending on whether the individual had skin injuries or not. *S. suis* infection occurred predominantly in males but controls were not matched by gender. As a sensitivity analysis, we therefore repeated the multivariate analysis including only male cases and controls. All analyses were performed with Stata version 10.1 (StataCorp) software.

Results

Between May 2006 and June 2009, 722 patients with suspected CNS infections or severe malaria were admitted to HTD. *S. suis* meningitis was diagnosed in 108 patients. Seven cases were excluded as they did not meet the inclusion criteria. We also excluded 311 other patients as they did not meet the inclusion criteria for hospital controls (Figure 2). It was not possible to recruit community controls at the residency of one *S. suis* case because of the distance from the study site (Central Viet Nam, 800 kms from Ho Chi Minh City). During the period of recruitment we therefore included 101 cases of confirmed *S. suis* meningitis, 303 hospital controls and 300 community controls for analysis (Figure 2).

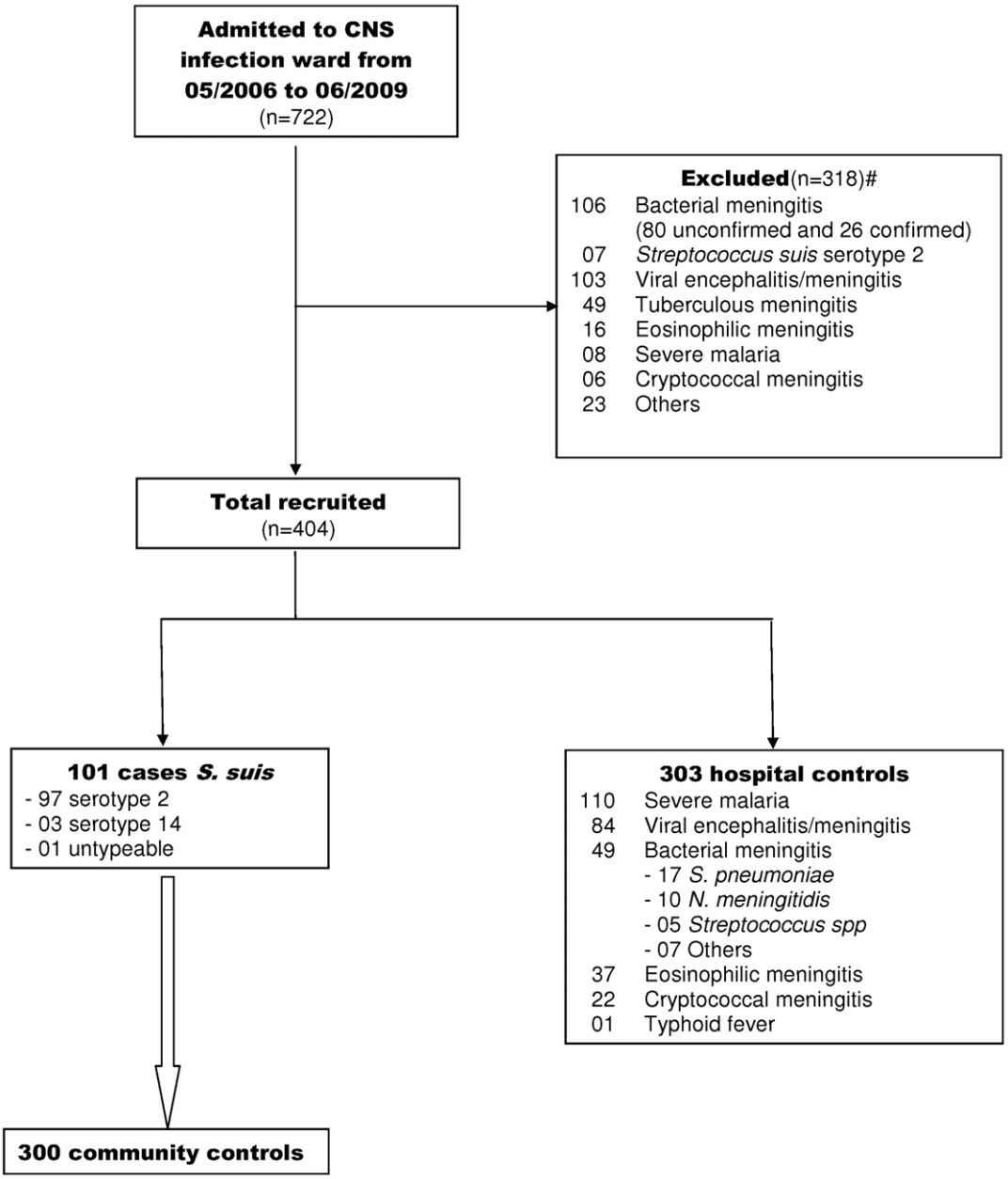

Figure 2. Flow diagram of inclusion of study participants. #**Reasons for exclusions of cases:** HIV (+) (3), transfer to other hospital because of presumed tuberculous meningitis (2), confusion more than 14 days after admission (1), language differences precluding interview (1). #**Reasons for exclusion of controls:** death (37), prolonged coma (72), unconfirmed bacterial meningitis (80), transfer to other hospitals (61), use of antimicrobial agents for more than 2 days in case of suspected viral encephalitis/meningitis (38), and absence of diagnosis of CNS infection (23). For one case, community controls could not be included because of too long distance of community to study site.
doi:10.1371/journal.pone.0017604.g002

Characteristics of the participants

S. suis infection occurred sporadically throughout the year without any clear seasonality (Figure 3). Clustering of cases was not observed. Ninety-seven patients (96%) were infected with S. suis serotype 2 while only four patients (4%) were infected with other serotypes, including serotype 14 (3 cases) and untypeable serotype (1 case). *S. suis* cases were predominantly male (82%) and from a rural residence (81%) with a median (IQR) age of 50 (41–59) years. Twenty-one percent of cases had an occupation related to pigs, other exposure to pigs (46%), or reported eating "high risk" dishes in the two weeks prior to admission (48%). Hospital and community controls were more frequently female and (unmatched) hospital controls were significantly younger, with a higher proportion of urban residence (Table 3). We further analyzed the age distribution of cases, hospital controls and non-*S. suis* bacterial meningitis patients amongst the hospital controls. Nearly 70% of *S. suis* meningitis patients were older than 45 years, while only 25% of non-*S. suis* meningitis patients and 20% of all hospital controls belonged to this age group (Table 4). The 49 hospital controls with bacterial meningitis (not *S. suis*) were significantly younger than *S. suis* cases with median (IQR) age of 27 (23–45) compared to 50 (41–59) years ($p<0.001$).

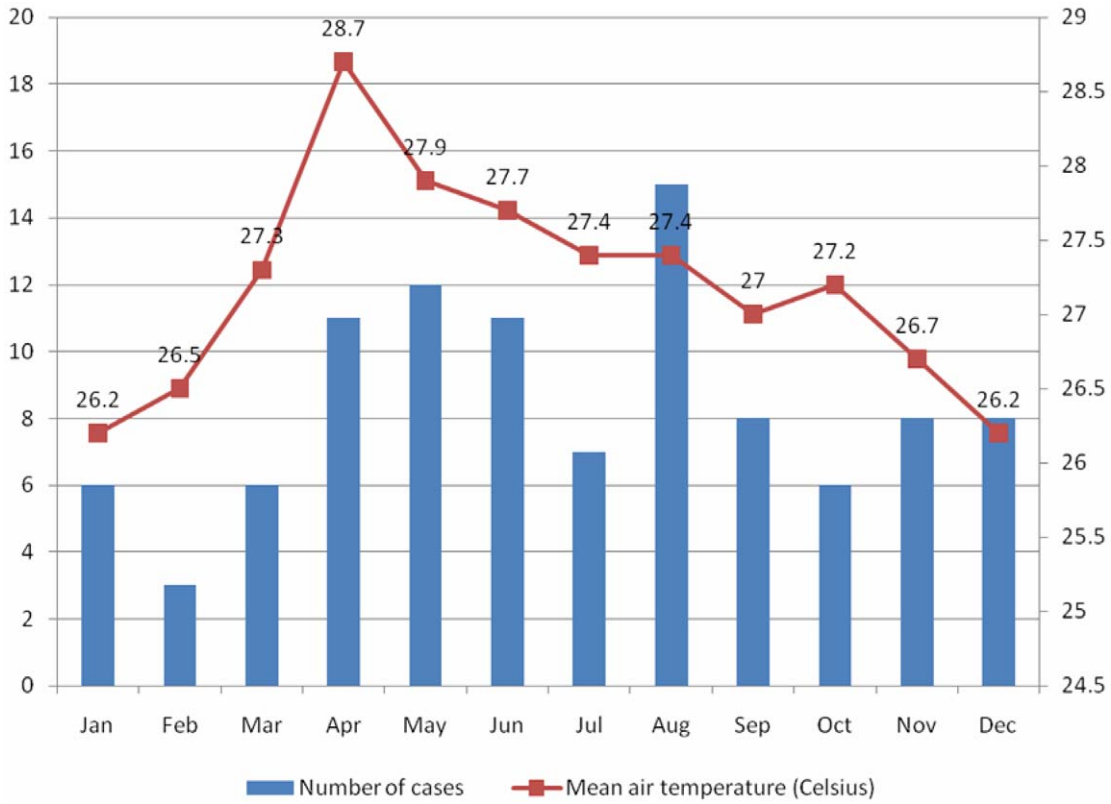

Figure 3. Distribution of *Streptococcus suis* meningitis cases during the study period. Distribution of *Streptococcus suis* meningitis cases and mean air temperature of southern Viet Nam in months during the study period (2006–2009) [17].
doi:10.1371/journal.pone.0017604.g003

Analysis of risk factors

Occupations related to pigs, breeding pigs at home, exposures to pigs or pork with skin injuries, eating "high risk" dishes in the last 2 weeks and having ill pigs at home in the last 4 weeks were associated with *S. suis* meningitis, after adjustment for residency, age and sex (Table 5). *S. suis* infection was independently associated with occupations related to pigs, exposures to pigs or pork in the presence of skin injuries in the 2 weeks prior to infection, and eating "high risk" dishes in the 2 weeks prior to infection after multivariate analysis. These associations were found in comparisons of cases with the hospital control group as well as with the community control group. Breeding pigs at home, diabetes mellitus, alcoholism or exposure to pigs or pork without skin injuries were not associated with *S. suis* infection in multivariate analysis (Table 6). In a sensitivity analysis, which included only male cases and controls, exactly the same risk factors were significant with similar odds ratios as in the main analysis. Risk factors identified by multivariate analysis of male cases and controls from either control group included eating "high risk" dishes ($OR_1 = 3.46$; 95%CI = [1.65–7.28] and $OR_2 = 4.79$; 95%CI = [2.02–11.40]), occupations related to pigs ($OR_1 = 6.33$; 95%CI = [1.55–25.79] and $OR_2 = 7.46$; 95%CI = [1.56–35.74]), and exposures to pigs or pork in the presence of skin injuries ($OR_1 = 5.81$; 95%CI = [1.07–31.48] and $OR_2 = 7.11$; 95%CI = [1.00–50.54]).

"High risk" dishes predominantly consisted of undercooked food (Table 3). Exposure to pigs or pork in the presence of skin injuries, eating "high risk" dishes, or both, were reported in 72/101 *S. suis* cases (71.3%), 91/303 hospital controls (30%) and 84/300 community controls (28%). For 26 *S. suis* cases (25.7%), compared to 52 hospital controls (17.2%) and 29 community controls (9.7%), eating these "high risk" dishes was the only risk factor reported.

Human carriage

To investigate potential carriage of *S. suis* serotype 2, 197 throat swab samples and 197 rectal swab samples were taken from 101 *S. suis* patients (Table 7). Six patients had PCR positive results, including one throat sample and six rectal samples. *S. suis* serotype 2 was cultured from this throat sample and from one of these rectal samples. Three of these six patients had pigs at home. No illness was reported in the last 4 weeks in these pigs, and the PCR results of pig tonsil swab samples were negative. None of these patients had skin injuries. Three patients had eaten pig intestines prior to admission. Of these, the throat swab sample was positive in one patient and rectal swab samples were positive in the two others. One patient, who had eaten pig intestines two days before admission, had two PCR positive rectal swab samples on separate occasions. *S. suis* serotype 2 was cultured from the first sample.

We collected 1162 throat and rectal swab samples from the 303 hospital controls and 4492 throat and rectal swab samples from healthy persons, including 300 community controls and 920 household members of cases, hospital controls and community controls. In none of these samples was S. *suis* serotype 2 detected. (Table 7).

Pig carriage

We collected 571 pig swab samples from pigs present around the house of 22 of 23 cases, 28 of 41 community controls and 13 of

Table 3. Characteristics of *Streptococcus suis* cases and controls.

Characteristics	Cases (n = 101)	Hospital controls (n = 303)	Community controls (n = 300)
Sex, n(%)			
Male	83 (82.2)	202 (66.7)	169 (56.3)
Female	18 (17.8)	101 (33.3)	131 (43.7)
Residence, n(%)			
Rural	82 (81.2)	193 (63.7)	243 (81)
Urban	19 (18.8)	110 (36.3)	57 (19)
Age (years), median (interquartile range)	50 (41,59)	27 (20,40)	50 (41,6)
Occupations related to pigs[1], n(%)	21 (20.79)	8 (2.64)	8 (2.7)
Education level, n(%)			
Primary	52 (51.5)	96 (31.7)	175 (58.3)
Secondary "level 2"	30 (29.7)	134 (44.2)	73 (24.3)
Secondary "level 3"	11 (10.9)	40 (13.2)	30 (10)
University	1 (1)	21 (6.9)	2 (0.7)
Illiterate	6 (5.9)	12 (4)	19 (6.3)
Religion			
Buddhism	64 (63.4)	160 (52.8)	181 (60.3)
Catholicism	12 (11.8)	42 (13.9)	31 (10.3)
Christianity	1 (1)	4 (1.3)	0
Cao Dai	7 (7)	17 (5.6)	29 (9.7)
Other	2 (2)	1 (0.3)	6 (2)
No	15 (15)	77 (25.4)	53 (17.7)
Ethnic backgroun			
Kinh	99 (98.0)	287 (94.7)	298 (99.3)
Khmer	1 (1)	4 (1.3)	2 (0.7)
Chinese	1 (1)	4 (1.3)	0
Other	0	8 (2.6)	0
Medical history, n(%)			
Diabetes mellitus	3 (3)	3 (1)	4 (1.3)
Alcoholism	14 (13.9)	18 (5.9)	20 (6.7)
Splenectomy	1 (1	0	0
Skin injuries, n(%)	33 (32.7)	18 (5.9)	11 (3.7)
Breeding pigs at home, n(%)	23 (22.8)	33 (10.9)	41 (13.7)
Any exposure to pigs/pork in the last 2 weeks, n(%)	46 (45.5)	39 (12.9)	55 (18.3)
With skin injuries	20 (19.8)	5 (1.7)	2 (0.7)
Without skin injuries	26 (25.7)	34 (11.2)	53 (17.7)
Eating any "high risk" dish in the last 2 weeks	48 (47.5)	66 (21.8)	48 (16.)
Fresh pig blood	5 (5)	5 (1.7)	5 (1.7)
Tonsils/tongue	19 (18.8)	29 (9.6)	25 (8.3)
Stomach/intestines	45 (44.6)	53 (17.5)	42 (14)
Uterus	8 (7.9)	8 (2.6)	13 (4.3)
Undercooked pig blood	11 (10.9)	18 (5.9)	5 (1.7)
Ill pigs at home in the last 4 weeks, x/n (%)	10/23 (43.5)	1/33 (3.0)	0/40 (0)
Pigs at home with *S. suis* serotype 2 (confirmed by PCR), x/n[2] (%)	9/22 (40.9)	3/13 (23.1)	5/28 (17.9)

[1]Butcher, pig breeder, slaughterer, roaster, meat transporter, meat processing, veterinarian and cook.
[2]Number of households with any number of PCR positive pig swab samples/ number of households where pigs where present and samples were taken.
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33 hospital controls respectively, who kept pigs around the house (Table 3). The median herd size was 7 pigs (range, 1 to 50). *S. suis* serotype 2 was detected in 9 (41%) case group herds, 3 (23%) hospital control group herds, and 5 (18%) matched community control group herds. Differences between case group and control groups were not statistically significant (Table 5).

Table 4. Age distribution of *Streptococcus suis* cases and hospital controls.

Age groups	Cases	Hospital controls	BM[1] (not *S. suis*) in hospital controls
<30	5 (5)	173 (57.1)	27 (55.1)
30–44	29 (28.7)	78 (25.7)	9 (18.4)
45–59	45 (44.6)	33 (10.9)	8 (16.3)
60–74	15 (14.9)	16 (5.3)	3 (6.1)
75+	7 (6.9)	3 (1)	2 (4.1)

[1]Bacterial meningitis.

doi:10.1371/journal.pone.0017604.t004

Discussion

We conducted the largest prospective epidemiological assessment of risk factors of *S. suis* infection globally. In addition to the previously suggested risk factors, occupational exposure and contact with pigs or pork without skin protection, we identified the ingestion of food with a high risk of contamination with *S. suis* serotype 2 to be an important risk factor for *S. suis* meningitis. To investigate eating habits as a risk factor of *S. suis* infection, we focused on potential "high risk" dishes common in Vietnam. These include fresh or under-cooked blood, tonsils, tongue, stomach, intestines and uterus. Such food items typically are undercooked when eaten as a main dish (as opposed to as components of well cooked main dishes such as rice or noodle soups), as was generally the case in our patients. By multivariate analysis, we demonstrated that eating these "high risk" dishes in the 2 weeks prior to admission was a significant risk factor for this infection. Eating habits were also confirmed as a risk factor during a relatively small *S. suis* outbreak in Thailand, associated with consumption of fresh pig blood. [12]. Eating pork was not associated with *S. suis* cases in a matched case-control study conducted during the outbreak in Sichuan province in 2005 [13]. *S. suis* lives as normal flora in the respiratory, gastrointestinal and genital tract of pigs and can cause invasive disease in

Table 5. Risk factors of *Streptococcus suis* infection on univariate analysis.

Exposure	Cases versus Hospital controls				Cases versus Community controls			
	OR[1] (95%CI)	p value	OR[2] (95%CI)	p value	OR[1] (95%CI)	p value	OR[3] (95%CI)	p value
Occupations related to pigs	9.68 (4.13–22.67)	<0.001	7.51 (2.85–19.82)	<0.001	11.50 (4.31–30.65)	<0.001	11.01 (4.03–30.12)	<0.001
Medical history								
Diabetes mellitus	3.06 (0.61–15.41)	0.175	0.82 (0.13–5.23)	0.830	2.25 (0.50–10.05)	0.288	3.75 (0.75–18.73)	0.107
Alcoholism	2.55 (1.22–5.33)	0.013	1.31 (0.54–3.16)	0.547	2.50 (1.15–5.45)	0.021	1.48 (0.63–3.31)	0.381
Skin injuries	7.68 (4.08–14.46)	<0.001	8.16 (3.72–17.92)	<0.001	22.09 (7.79–62.64)	<0.001	22.30 (7.55–65.84)	<0.001
Breeding pigs at home	2.41 (1.34–4.35)	0.003	2.34 (1.09–5.00)	0.028	1.95 (1.04–3.65)	0.036	1.99 (1.04–3.80)	0.036
Any exposure to pigs/pork in the last 2 weeks	5.66 (3.38–9.49)	<0.001	4.69 (2.43–9.07)	<0.001	4.51 (2.55–7.97)	<0.001	4.16 (2.30–7.52)	<0.001
With skin injuries	14.72 (5.36–40.42)	<0.001	12.16 (3.74–39.50)	<0.001	30 (7.01–128.35)	<0.001	26.95 (6.14–118.23)	<0.001
Without skin injuries	2.74 (1.55–4.86)	0.001	2.06 (0.99–4.27)	0.052	1.66 (0.92–3.00)	0.090	1.57 (0.85–2.91)	0.152
Eating any "high risk" dish in the last 2 weeks	3.25 (2.02–5.24)	<0.001	2.48 (1.35–4.52)	0.003	6.00 (3.33–10.81)	<0.001	4.38 (2.72–8.08)	<0.001
Ill pigs at home in the last 4 weeks[4]	24.62 (2.85–212.24)	0.004	30.10 (2.72–333.64)	0.006	-	-	-	-
Pigs at home with *S. suis* serotype 2 (confirmed by PCR)[5]	2.31 (0.49–10.82)	0.289	7.83 (0.68–90.19)	0.099	-	-	-	-

[1]Crude OR based on logistic (hospital controls) or conditional logistic regression (community controls).
[2]Adjusted for age, sex and rural/urban residence, using logistic regression.
[3]Adjusted for sex (matched for age and residence), using conditional logistic regression.
[4]Only individuals with pigs at home were analyzed. OR could not be analyzed for community controls because none of them reported ill pigs at home.
[5]Only individuals who had pig swab samples at their houses were analyzed. OR could not be analyzed for community controls because there was no discordant pairs included in the analysis.

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Table 6. Risk factors of *Streptococcus suis* infection - multivariate analysis.

Exposure	Cases versus Hospital controls		Cases versus Community controls	
	OR (95%CI)	p value	OR (95%CI)	p value
Occupations related to pigs	3.84 (1.32–11.11)	0.013	5.52 (1.49–20.39)	0.010
Medical history				
Diabetes mellitus	1.10 (0.17–7.31)	0.918	4.11 (0.78–21.68)	0.095
Alcoholism	1.02 (0.38–2.73)	0.969	0.72 (0.24–2.14)	0.553
Breeding pigs at home	1.02 (0.39–2.69)	0.965	0.83 (0.34–2.03)	0.681
Any exposure to pigs/pork in the last 2 weeks				
With skin injuries	7.48 (1.97–28.44)	0.003	15.96 (2.97–85.72)	0.001
Without skin injuries	2.15 (0.88–5.24)	0.092	1.14 (0.49–2.69)	0.757
Eating any ''high risk'' dish in the last 2 weeks	2.22 (1.15–4.28)	0.017	4.44 (2.15–9.15)	<0.001
Rural	2.39 (1.13–5.04)	0.022	-	-
Age (by +10 years)	2.59 (2.04–3.29)	<0.001	-	-
Male sex	4.47 (1.88–10.64)	0.001	3.53 (1.59–7.82)	0.002

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pigs. *S. suis* was isolated from raw pork samples obtained from markets in Hong Kong [14]. There may be a high bacterial load in food items that are kept at high ambient temperatures. Therefore, patients may be infected with *S. suis* through gastrointestinal tract if the ''high risk'' dishes are served as raw or under-cooked food.

We observed that the mean age of *S. suis* meningitis patients was significantly higher than the age of patients with bacterial meningitis caused by other bacteria, and that high age was associated with increased risk of infection with *S. suis*. In contrast, pediatric infections with *S. suis* are extremely rare, presumably related to a lack of exposure associated with increased risk of *S. suis* infection, in children.

The association between human *S. suis* infection and occupational exposures to pigs or pork has been reported in Europe and Asia since 1968 [6,15,16]. In Vietnam, the proportion of patients reported to have occupational exposures was lower than reported in European patients but it remained an important independent risk factor. Slaughtering and processing sick or dead pigs were also associated with *S. suis* infection in a case-control study conducted during the Sichuan outbreak [13].

Significant skin injury was evident in 5/35 (14%) of people with *S. suis* in the UK, 4/15 cases (16%) in Hong Kong and 104/215 cases (48%) in Sichuan province's outbreak [2,7,15]. In our study, skin injuries were reported in 33/101 (33%) of *S. suis* patients compared to 18/303 (6%) of hospital controls and 11/300 (4%) of community controls (Table 3). These minor skin injuries may allow direct entry of the bacteria in people with direct contact with infected pigs or pork. Contact with pigs within the last two weeks in the presence of skin lesions was associated with a significant high risk of infection (Table 6). Skin injuries were most often recorded in slaughter house workers, cooks and housewives involved in processing meat. Skin protection, including gloves, hand washing and exclusion of people with obvious skin lesions from direct contact with pigs and pork meat, may help to reduce the incidence of the disease.

We were unable to demonstrate *S. suis* serotype 2 carriage in 1522 healthy persons or patients without *S. suis* infection, including those with pig exposures. In contrast, 6/101 (6%) of patients had PCR positive swab samples, a rate similar to what was found in slaughterhouse workers in Germany [16]. Eating pig intestines in the few days prior to admission was reported in 3/6 of the patients with a PCR positive throat or rectal swab, two of which were also culture positive. Taken together, rather than indicating human carriage of *S. suis* serotype 2, our results strengthen the hypothesis that the gastrointestinal tract may be a route of entry for at least a proportion of patients.

In conclusion, *S. suis* is an important and emerging public health issue in Asia and one with the potential for both endemic transmission and for explosive epidemics. We identified risk factors for *S. suis* infection which can be addressed in health education programs targeted at individuals and communities at risk, focusing on skin protection for those in direct contact with pigs or pork and avoiding eating raw or under-cooked pig products.

Table 7. Results of *Streptococcus suis* serotype 2 PCR of throat and rectal swabs.[1]

Samples	Cases		HM[2]/cases		Hospital controls		HM/hospital controls		Community controls		HM/community controls	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Throat	1/101	0/96	0/205	0/182	0/302	0/279	0/291	0/212	0/300	0/291	0/424	0/347
Rectum	5/101	1/96	0/204	0/181	0/302	0/279	0/291	0/211	0/300	0/291	0/422	0/340

[1]Number positive/total number tested (each person had 2 samples taken on 2 separate occasions with a minimum of 10–14 days in between).
[2]Household members.
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Author Contributions

Conceived and designed the experiments: HDTN HT TTH JF BGS CS. Performed the experiments: LTPT NVMH TVTN LTPT TSD JC NTH. Analyzed the data: NHDT HTTH MW BGS JF CS. Wrote the paper: HDTN MW JF TTH BGS CS. Recruitment of patients: CQT NHP TTHC DXS NVVC NTC. Sampling of animals: NVD.

References

1. Ma Y, Feng Y, Liu D, Gao GF (2009) Avian influenza virus, Streptococcus suis serotype 2, severe acute respiratory syndrome-coronavirus and beyond: molecular epidemiology, ecology and the situation in China. Philos Trans R Soc Lond B Biol Sci 364: 2725–2737.
2. Yu H, Jing H, Chen Z, Zheng H, Zhu X, et al. (2006) Human Streptococcus suis outbreak, Sichuan, China. Emerg Infect Dis 12: 914–920.
3. Nguyen TH, Tran TH, Thwaites G, Ly VC, Dinh XS, et al. (2007) Dexamethasone in Vietnamese adolescents and adults with bacterial meningitis. N Engl J Med 357: 2431–2440.
4. Mai NT, Hoa NT, Nga TV, Linh le D, Chau TT, et al. (2008) Streptococcus suis meningitis in adults in Vietnam. Clin Infect Dis 46: 659–667.
5. Wertheim HF, Nguyen HN, Taylor W, Lien TT, Ngo HT, et al. (2009) Streptococcus suis, an important cause of adult bacterial meningitis in northern Vietnam. PLoS One 4: e5973.
6. Arends JP, Zanen HC (1988) Meningitis caused by Streptococcus suis in humans. Rev Infect Dis 10: 131–137.
7. Kay R, Cheng AF, Tse CY (1995) Streptococcus suis infection in Hong Kong. Qjm 88: 39–47.
8. Wisselink HJ, Reek FH, Vecht U, Stockhofe-Zurwieden N, Smits MA, et al. (1999) Detection of virulent strains of Streptococcus suis type 2 and highly virulent strains of Streptococcus suis type 1 in tonsillar specimens of pigs by PCR. Vet Microbiol 67: 143–157.
9. Corless CE, Guiver M, Borrow R, Edwards-Jones V, Fox AJ, et al. (2001) Simultaneous detection of Neisseria meningitidis, Haemophilus influenzae, and Streptococcus pneumoniae in suspected cases of meningitis and septicemia using real-time PCR. J Clin Microbiol 39: 1553–1558.
10. Nga TVT, Nghia HD, Tu LTP, Diep TS, Mai NT, et al. Real-time PCR for detection of Streptococcus suis serotype 2 in cerebrospinal fluid of human patients with meningitis. Diagn Microbiol Infect Dis, (in press).
11. Harrell F (2001) Regression Modeling Strategies With Applications to Linear Models, Logistic Regression, and Survival Analysis. New York: Springer.
12. Naretrit Khadthasrima HT, Thammawitjaya P, Pingsusean D, Akkanij B, Jaikhar A Human Streptococcus suis outbreak in Phayao province, Thailand, 2007.
13. Yu HJ, Liu XC, Wang SW, Liu LG, Zu RQ, et al. (2005) [Matched case-control study for risk factors of human Streptococcus suis infection in Sichuan Province, China]. Zhonghua Liu Xing Bing Xue Za Zhi 26: 636–639.
14. Ip M, Fung KS, Chi F, Cheuk ES, Chau SS, et al. (2007) Streptococcus suis in Hong Kong. Diagn Microbiol Infect Dis 57: 15–20.
15. Walsh B, Williams AE, Satsangi J (1992) *Streptococcus suis* type 2: pathogenesis and clinical disease. Review in Medical Microbiology 3: 65–71.
16. Strangmann E, Froleke H, Kohse KP (2002) Septic shock caused by Streptococcus suis: case report and investigation of a risk group. Int J Hyg Environ Health 205: 385–392.
17. Statistical Yearbook of Viet Nam 2008. Ha Noi: Statistical Publishing House.819 p.

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Real-time PCR for detection of *Streptococcus suis* serotype 2 in cerebrospinal fluid of human patients with meningitis

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Abstract

Streptococcus suis serotype 2 is an emerging zoonotic pathogen and is the main cause of acute bacterial meningitis in adult patients in Vietnam. We developed an internally controlled real-time PCR for detection of *S. suis* serotype 2 in cerebrospinal fluid (CSF) samples targeted at the *cps2J* gene. Sensitivity and specificity in culture-confirmed clinical samples were 100%. The PCR detected *S. suis* serotype 2 infection in 101 of 238 (42.4%) prospectively collected CSF samples, of which 55 (23%) were culture positive. Culture-negative but PCR-positive CSF samples were significantly associated with the use of antimicrobial agents before admission. *S. suis* serotype 2 infection was more common than infections with *Streptococcus pneumoniae* and *Neisseria meningitidis* combined. Our results strikingly illustrate the additional diagnostic value of PCR in patients who are pretreated with antimicrobial agents and demonstrate the extremely high prevalence of *S. suis* infections among Vietnamese adult patients with bacterial meningitis.

Keywords: Bacterial meningitis; *Streptococcus suis* serotype 2; CSF; Real-time PCR

1. Introduction

Streptococcus suis is an emerging zoonotic human pathogen. *S. suis* infection is acquired through exposure to contaminated pigs or pig meat. Healthy pigs can carry multiple serotypes of *S. suis* in their nasal cavities, tonsils, and upper respiratory, genital, and alimentary tracts. Based on differences in antigenic properties of the polysaccharide capsule, 33 serotypes have been distinguished to date, of which only a limited number are responsible for infections in pigs, including serotypes 1 to 9 and 14. Serotype 2 is considered to be the most pathogenic for both humans and pigs and is the single most common serotype found in human infection (Gottschalk et al., 2007).

Over the past few years, the number of reported *S. suis* infections in humans has increased substantially, with most cases originating in Southeast Asia where there is a high density of pigs. Increased awareness, particularly following the occurrence of an outbreak of human and pig infection in the Sichuan province in China in 2005, has likely contributed to this increase in reported human infections (Tang et al., 2006; Ye et al., 2006). Meningitis and septicemia are the most common clinical manifestations of human *S. suis* infection; hearing loss is a frequent complication (Wertheim et al., 2009a).

Although *S. suis* can be cultured from cerebrospinal fluid (CSF) or blood samples with use of standard microbiological

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techniques, infection often goes undiagnosed or positive cultures are misidentified as *Streptococcus* species, alpha-hemolytic or viridans streptococci, *Enterococcus faecalis*, *Aerococcus viridans*, or *Streptococcus pneumoniae* (Donsakul et al., 2003; Lutticken et al., 1986). Furthermore, culture results can be negative as a result of antibiotic use before the collection of specimens.

During a randomized placebo-controlled clinical trial on the adjuvant use of dexamethasone in adult patients with bacterial meningitis, carried out at the Hospital for Tropical Diseases in Ho Chi Minh City, Vietnam, *S. suis* serotype 2 was found to be the most common pathogen isolated from CSF cultures (Nguyen et al., 2007). As up to 60% of patients had used antimicrobial agents before submission to the hospital, and culture results were negative for 50% of patients (Nguyen et al., 2007), an internally controlled diagnostic real-time PCR was set up for detection of *S. suis* serotype 2 to further study the importance of this pathogen in patients with bacterial meningitis in this region. Here we report the design of this method and its prospective evaluation.

2. Materials and methods

2.1. Sample collection

This study was performed at the Hospital for Tropical Diseases, a tertiary referral hospital for infectious diseases. CSF samples were collected and stored as part of a randomized placebo-controlled clinical trial carried out at the Hospital for Tropical Diseases between November 1996 and May 2005 (Nguyen et al., 2007). CSF samples were sent for biochemical and microbiological investigations, and an aliquot was stored at −70 °C in a dedicated freezer on the ward. These samples were studied retrospectively. CSF samples were prospectively collected from all consecutive adult patients (age ≥15 years) presenting with fever and neck stiffness and/or altered consciousness at the Hospital for Tropical Diseases from May 2006 until June 2009, and were aliquoted immediately after lumbar puncture on the ward. Aliquots were sent to the biochemistry and microbiology laboratories for immediate processing and analyses. A separate aliquot was sent to the molecular diagnostics laboratory, where samples where stored for a maximum of 48 h at −70 °C until testing (prospective study). Standard measures for prevention of PCR contamination are operational at the molecular diagnostic laboratory, including a unidirectional workflow in physically separated laboratories for reagent preparation, nucleic acid extraction, and amplification and analysis, respectively.

Demographic, clinical, and laboratory data were recorded for all patients. The study was approved by the ethical review boards of the Hospital for Tropical Diseases and the University of Oxford (OXTREC).

2.2. Bacterial culture

All CSF samples were spun down and a Gram stain was made. The pellet was inoculated on blood and chocolate agar plates and in brain heart infusion broth for enrichment. Plates were incubated at 37 °C in 5% CO_2 for 18 h. The broth was incubated aerobically and subcultured if growth was present. Bacteria were identified using standard identification methods. *S. suis* was identified on the basis of colony morphology, negative catalase reaction, optochin resistance, and APIStrep (Biomerieux, Ho Chi Minh City, Vietnam). Serotyping was performed by slide agglutination with use of specific antisera (Statens Serum Institute, Copenhagen, Denmark).

Blood culture was performed using the BACTEC 9050 system, and positive culture results were identified as described above.

2.3. Primers and probes

Primers and probe for *S. suis* serotype 2 real-time PCR were designed using Primer Express Software and BLAST analysis, and were targeted at the *cps2J* gene (Smith et al., 1999, 2000), which is part of the operon encoding the serotype 2 and serotype 1/2 specific polysaccharide capsule of *S. suis*. Primers cps2JF (GGTTACTTGCTACTTTTGATGGAAATT) and cps2JR (CGCACCTCTTTTATCTCTTCCAA) and probe (FAM-TCAAGAATCTGAGCTGCAAAAGTGTCAAATTGA-TAMRA) were used for amplification and detection of an 88-bp amplicon. Primers and probes for real-time PCR for detection of *S. pneumoniae*, *Haemophilus influenzae* type b, and *N. meningitidis* were as described by Corless et al. (2001) except that for all probes, FAM (6-carboxyfluorescein) and TAMRA were used as reporter and quencher, respectively.

Primers and probe for detection of internal control (IC) DNA (see below) were as described by van Doornum et al. (2003). The IC probe was labeled with Cy5 and BHQ1.

2.4. Internal control

The efficiency of the DNA extraction and the amplification during the PCR was monitored using an IC, consisting of a pretest determined concentration of Phocid herpesvirus. IC was added to all samples before DNA extraction, as described by van Doornum et al. (2003). Concentration of IC was such that after efficient extraction and amplification, a Cy5 cycle threshold value (Ct value) between 33 and 37 should be expected for the IC-specific PCR reaction. Higher Ct values or negative results were interpreted as loss of DNA during extraction or inhibition of the PCR assay, in which case extraction and amplification were repeated. PhHV was kindly provided by M. Schutten (EMC, Rotterdam, the Netherlands).

2.5. DNA extraction of pure cultures and CSF samples

A 100-μL aliquot of a bacterial suspension or of unspun CSF was treated with 0.1 volume of prelysis buffer (1% SDS, 5% Tween 20, and 5% Sarkosyl in 1× TE) at 37 °C for

1 h. A 20-μL volume of IC at a predetermined concentration was added to the sample, and DNA was extracted by manual extraction (retrospective study) or automated extraction using the EasyMag extraction system (BioMerieux, Ho Chi Minh City, Vietnam), according to manufacturer's instructions (prospective study). The manual extraction was performed as described by Boom et al. (1999), using lysis buffer L7 that contains 1 mg/mL α-casein. The DNA was eluted in a final volume of 100 μL.

2.6. PCR components and amplification

The final PCR volume was 25 μL. The PCR mix consisted of 5 mmol/L $MgCl_2$, 0.2 mmol/L each deoxynucleoside triphosphates dATP, dCTP, dGTP, dUTP, and 1 U of Hot Start Taq DNA polymerase (Qiagen, Hanoi, Vietnam) to which 5 μL of extracted DNA was added. Final concentrations of the 2 primers and probe sets for target and IC were 0.4 μmol/L of each primer and 0.1 μmol/L of each probe. PCR amplification conditions consisted of 15 min at 95 °C and 45 cycles of 30 s at 95 °C, 30 s at 60 °C, and 30 s at 72 °C in a Chromo 4 Real-time PCR system (Biorad, Ho Chi Minh City, Vietnam). Negative (no-template) controls of both extraction and PCR were included in each run.

The PCR was considered positive if negative controls were all negative and a FAM signal with a Ct value of ≤40 could be obtained from the sample. A PCR was considered negative if negative controls were all negative, and the IC showed a Cy5 Ct value within the expected range, and a FAM signal could not be obtained from the sample or the Ct value was >40. Any PCR that yielded a FAM Ct value >35 was repeated in duplicate for confirmation. The PCR was considered indeterminate if the IC showed a Cy5 Ct value outside of the expected range and a FAM signal could not be obtained or the Ct value was >40, in which case DNA extraction and the PCR were repeated as described before.

The sensitivity and specificity of the PCR assay were determined on the basis of PCR results in bacterial culture-confirmed samples. PCR for *S. suis* serotype 2 was run on all culture-positive samples, including those growing *S. suis*, *S. pneumoniae*, *N. meningitidis*, *H. influenzae*, or other pathogens (Tables 2 and 3).

2.7. Analytical sensitivity

To determine the detection limit of the assay, including the DNA extraction, a 10-fold serial dilution of a 0.5 McFarland suspension of *S. suis* serotype 2 strain 31533, kindly provided by M. Gottschalk (Montreal, Canada), was prepared in Todd Hewitt Broth. Fifty microliters of each dilution was spread out on blood agar plates in triplicate and incubated at 37 °C in 5% CO_2 overnight for colony counting. A 100-μL volume of each dilution was used for DNA extraction in triplicate, in the absence and presence of IC. Five microliters of DNA was used for real-time PCR, as described above.

Table 1
Comparative analytical sensitivities of *S. suis* serotype 2 primers in the presence and absence of IC DNA

IC DNA	Mean Ct value at the following bacterial concentration		
	10^5 CFU/mL	10^4 CFU/mL	10^3 CFU/mL
Present	29.99	33.25	36.78
Absent	30.21	33.19	36.80

3. Results

The PCR assay detected *S. suis* serotype 2 at a concentration of 2×10^2 colony-forming units (CFUs) per milliliter, resulting in an analytical sensitivity of 1–5 CFUs per reaction. This analytical sensitivity did not vary in the presence or absence of IC DNA (Table 1).

Sensitivity of the PCR was 100% when tested against 114 stored samples from culture-confirmed cases of meningitis with *S. suis* serotype 2 (Table 2A). The PCR was negative in

Table 2
Sensitivity and specificity of *S. suis* serotype 2 PCR on culture-confirmed CSF samples, retrospective study (A) and prospective study (B)

A.	No. of samples	PCR positive	PCR negative
S. suis serotype 2	114	114	0
S. suis serotype 14	1	0	1
N. meningitidis	11	0	11
Neisseria species	1	0	1
S. pneumoniae	50	0	50
H. influenzae	5	0	5
Streptococcus bovis	2	0	2
Streptococcus agalactiae	1	0	1
Streptococci, viridans group	6	0	6
Staphylococcus aureus	2	0	2
Escherichia coli	8	0	8
Klebsiella pneumoniae	10	0	10
Proteus mirabilis	1	0	1
Pseudomonas aeruginosa	1	0	1
Total	213	114	99

B.	No. of samples	No. of PCR positive	No. of PCR negative
S. suis serotype 2	55	55	0
S. suis serotype 14	2	0	2
S. suis, untypeable	1	0	1
N. meningitidis	4	0	4
S. pneumoniae	16	0	16
H. influenzae	1	0	1
Streptococcus agalactiae	1	0	1
Nonhemolytic streptococci	1	0	1
Staphylococcus aureus	1	0	1
Escherichia coli	3	0	3
Klebsiella pneumoniae	2	0	2
Acinetobacter spp.	1	0	1
Enterococcus avium	1	0	1
Listeria spp.	4	0	4
Salmonella spp.	1	0	1
Total	94	55	39

Table 3
Demographic, clinical, and outcome characteristics of 248 patients included in prospective study

Characteristics	Frequency (%) $N = 248$
Age (years), median (interquartile range)	46.5 (34–59)
Sex, male	181 (73.0)
Occupation	
Farmer	79 (31.9)
Worker	35 (14.1)
Student	4 (1.6)
Seller	22 (8.9)
Occupation related to pigs	
Butcher	7 (2.8)
Slaughterer	6 (2.4)
Pig breeder	4 (1.6)
Housewife	14 (5.7)
Other	32 (12.9)
No job	45 (18.2)
Underlying diseases/predisposing factors	
Diabetes mellitus	19 (7.7)
Alcoholism	18 (7.3)
Otitis media	6 (2.4)
Splenectomy	2 (0.8)
Head trauma	26 (10.5)
Cardiovascular diseases[a]	17 (6.9)
Pig/pork exposures[b]	51 (20.6)
HIV/AIDS	4 (1.6)
Used intravenous antibiotics before admission	157 (63.3)

[a] Valvular heart diseases, atrial fibrillation.
[b] Occupation related to pig/pork, eating pig's intestines and fresh blood, etc.

all 99 samples that were culture-positive for other bacterial pathogens, including *S. suis* of other serotypes (100% specificity, Table 2A). All PCR-negative samples gave Ct values for the IC within the expected range.

Stored CSF samples that were culture positive for *S. pneumoniae*, *N. meningitidis*, and *H. influenzae* type b were also subjected to real-time PCR for specific detection of these pathogens. Of 50 samples culture positive for *S. pneumoniae*, 48 were positive in the PCR for detection of *S. pneumoniae* DNA, while all 11 samples culture positive for *N. meningitidis* and all 4 samples positive for *H. influenzae* type b were also positive in the respective specific PCRs.

During the study period, we admitted 248 consecutive patients with a clinical suspicion of bacterial meningitis. Demographic, clinical, and outcome characteristics of these 248 patients are shown in Table 3. CSF samples from 238 patients were prospectively studied using bacterial culture and real-time PCR for detection of *S. suis* serotype 2, *N. meningitidis*, *H. influenzae* type b, and *S. pneumoniae*. A lumbar puncture was contraindicated in one patient because of risk of brain herniation. CSF samples were erroneously not sent for PCR analysis for the remaining 9 patients.

All 55 *S. suis* serotype 2 culture-positive samples collected prospectively on admission were also positive in the *S. suis* serotype 2-specific PCR. The admission CSF sample of one patient with culture-confirmed *S. suis* serotype 2 meningitis was not available for PCR analysis and PCR result of the second CSF sample from this patient (collected

Table 4
Results of Gram stain, culture, and real-time PCR for detection of *S. suis* serotype 2, *S. pneumoniae*, *H. influenzae* type b, and *N. meningitidis* on CSF samples, and blood culture, prospective study

	No. of positive specimens				
	CSF Gram stain ($n = 247$)	CSF culture ($n = 247$)	CSF PCR ($n = 238$)	Blood culture ($n = 222$)	Total ($N = 248$)
S. suis serotype 2	50	61[a]	101[a]	34	107
S. suis serotype 14	1	2	0	1	2
S. suis untypeable	1	1	0	0	1
N. meningitidis	4	4	11	2	11
S. pneumoniae	9	16	37[b]	8	39
H. influenzae, nontypeable	0	1	0	1	1
Streptococcus bovis	1	1	nd[c]	0	1
Streptococcus agalactiae	2	1	0	2	2
β-Hemolytic streptococci	0	0	0	1	1
Nonhemolytic streptococci	0	1	0	1	1
Staphylococcus aureus	1	1	0	3	3
Escherichia coli	1	3	0	2	3
Klebsiella pneumoniae	2	4	0[d]	1	4
Acinetobacter spp.	0	1	0	0	1
Enterococcus avium	0	1	0	0	1
Listeria spp.	1	4	0	2	4
Salmonella spp.	0	1	0	0	1
Total	73	103	149	58	183

nd = not done.
[a] CSF samples were unavailable for PCR for 6 patients.
[b] *S. pneumoniae*-specific PCR was negative in 1 patient; CSF sample unavailable for PCR for 1 patient.
[c] CSF sample unavailable for PCR.
[d] CSF sample unavailable for PCR for 2 patients.

Table 5
Result of CSF investigations for *S. suis* serotype 2, *S. pneumoniae*, *N. meningitidis*

Antimicrobial agents before admission	Result of CSF investigations for *S. suis* serotype 2, *S. pneumoniae*, *N. meningitidis*			
	Culture positive, no. (%)	PCR positive, no. (%)	Median Ct value[a] (range)	*P* value[b]
Used	34/98 (34.7)	97/97 (100)	27.78 (15.39–38)	<0.001
Not used	39/47 (82.0)	42/43 (97.7)	24.66 (19.76–31.24)	
Unknown	8/11 (72.7)	10/11 (90.9)	24.57 (20.76–29)	

[a] Cycle treshold value of real-time PCR.
[b] Difference between Ct values for antimicrobial agents used versus not used, Wilcoxon rank-sum test.

after 5 days of antibiotic treatment) was negative. The *S. suis* serotype 2 specific PCR was negative in all 39 samples, which were culture confirmed with other bacterial pathogens, including *S. suis* of other serotypes (Table 2B).

S. suis was the most commonly identified pathogen (Table 4). PCR for *S. suis* serotype 2 was positive in 101 of 238 (42.4%) samples, of which 55 (23.1%) were culture positive. *S. pneumoniae* and *N. meningitidis* were detected in 37 (15.5%) and 11 (4.6%) patients of which 16 (6.7%) and 4 (1.7%) were culture positive, respectively. *Listeria* species were cultured from CSF of 4 patients. Infections with multiple bacterial species were not detected. All samples gave the expected results for the IC. Bacterial pathogens were detected in 183 of 248 (73.8%) adult patients suspected of bacterial meningitis when combining results of Gram stain, bacterial culture and PCR on CSF, and blood culture.

CSF was significantly more often culture negative in patients who were pretreated with antimicrobial agents before admission (Table 5). In contrast, detection rates by PCR were similar in patients who were pretreated and those who were not, although Ct values were significantly higher in patients who had received antimicrobial agents before collection of the CSF sample (Table 5). We compared characteristics between patients who were only positive by PCR in CSF and those of whom CSF or blood samples were also culture positive (Table 6). Patient characteristics related to exposure and clinical presentation were highly similar between the two groups, with the exception of a higher age and a higher prevalence of diabetes mellitus in patients who were PCR-positive only. In contrast, the median duration of illness was significantly longer and pretreatment with antimicrobial agents significantly more common in patients who were PCR-positive only. This was also reflected by lower CSF neutrophil counts, higher CSF glucose levels, and lower CSF lactate levels in the latter patients (Table 6).

4. Discussion

Human infections with *S. suis* are increasingly reported from various geographical areas. *S. suis* serotype 2 is the most common pathogen detected in adult patients with acute bacterial meningitis in Vietnam (Mai et al., 2008; Wertheim et al., 2009b). While *S. suis* is not difficult to culture on blood agar plates supplemented with 5% CO_2, CSF cultures may remain negative because of prior use of antimicrobial agents or low bacterial load. We developed a highly sensitive and specific real-time PCR for detection of *S. suis* serotype 2 in CSF. We designed primers targeted at the *cps2J* gene, which encodes a putative glycosyl transferase involved in the formation of the serotype 2 capsular polysaccharide. This gene was also used by other investigators as a target for conventional PCR for specific detection of *S. suis* serotype 2 in tonsillar and other pig samples (Wisselink et al., 2002). The *cps2J* gene is present in strains of serotype 2 and of

Table 6
Characteristics of patients with *S. suis* meningitis confirmed by CSF and/or blood culture and PCR, or only confirmed by PCR

Characteristics	CSF and/or blood culture and PCR positive (*n* = 67)	PCR positive (*n* = 43)	*P* value[a]
General information			
Age, median (IQR)	48 (38–56)	53 (45–61)	0.026
Male sex (*n*, %)	57 (85.1)	34 (79.1)	0.416
Residence (rural) (*n*, %)	53 (79.1)	32 (74.41)	0.567
Underlying diseases and exposure			
Diabetes mellitus (*n*, %)	1 (1.5)	6 (13.0)	0.010
Alcoholism (*n*, %)	8 (11.9)	2 (4.7)	0.194
Pig exposure (*n*, %)	26 (38.8)	14 (32.6)	0.506
Clinical manifestations			
Days of illness, median (IQR)	3 (3–5)	5 (4–7)	0.004
Antimicrobial therapy before admission (*n*, %)	35 (53.2)	38 (88.4)	<0.001
Fever (*n*, %)	65 (97.0)	42 (97.7)	0.522
Headache (*n*, %)	67 (100)	42 (97.7)	0.391
Nausea/vomiting (*n*, %)	49 (73.1)	27 (62.8)	0.252
Neck stiffness (*n*, %)	61 (91.0)	40 (93.0)	1.000
Glasgow Coma Score, median (IQR)	12 (9–14)	13 (9–15)	0.159
Tinnitus (*n*, %)	50 (74.6)	27 (62.8)	0.118
Deafness (*n*, %)	19 (28.4)	11 (25.6)	0.714
Skin injuries (*n*, %)	18 (26.9)	13 (30.2)	0.702
Herpes labialis (*n*, %)	29 (43.3)	21 (48.8)	0.568
Laboratory investigations median (IQR)			
▪ Blood			
White blood cells (10^3/L)	18 000 (12 400–23 000)	16 840 (12 600–23 550)	0.941
Neutrophil (%)	88 (83–91)	86.3 (80.3–90)	0.131
▪ CSF			
White cells (10^3/L)	1570 (760–3480)	1340 (340–2900)	0.153
Neutrophil (%)	86 (74–91)	70 (49–85)	0.002
Protein (g/L)	1.6 (1.3–2)	1.63 (1.2–2)	0.402
CSF/blood glucose ratio	0.24 (0.15–0.31)	0.40 (0.29–0.5)	<0.001
Lactate (mmol/L)	11.2 (6.8–15.7)	5.73 (4.4–8.3)	<0.001
Outcome (survival)	67 (100)	40 (100)[b]	

[a] Determined using Fisher exact test or Wilcoxon rank-sum test, as appropriate.
[b] Three patients were transferred to other hospitals and their outcomes are unknown.

serotype 1/2. Serotyping confirmed the presence of *S. suis* serotype 2 in all culture-positive CSF samples, and none contained serotype 1/2. To our knowledge, *S. suis* serotype 1/2 infection has never been reported in humans. So far, serotype 2 is the cause of more than 95% of reported human *S. suis* infections (Wertheim et al., 2009a), and only sporadic single cases of patients infected with *S. suis* serotypes 1, 4, and 16 have been described (Nghia et al., 2008; Wertheim et al., 2009a). However, one patient in our retrospective analysis and 2 patients in the prospective analysis were infected with *S. suis* serotype 14. As expected, these samples were negative in the PCR. While the absolute number of patients reported with *S. suis* serotype 14 infection is still very low, this serotype appears to contribute consistently to the infectious burden of *S. suis* as reported in Thailand (Kerdsin et al., 2009) and also observed in our study. While serotype 2 by far remains the predominant strain associated with human infection at present, inclusion of additional primer sets for detection of serotype 14 or generic detection of *S. suis* may therefore be considered in the future.

Our assay showed 100% sensitivity against samples that were culture positive for *S. suis* serotype 2, while analysis of CSF samples that were culture or PCR-positive for other pathogens than *S. suis* serotype 2, including *S. pneumoniae*, *N. meningitidis*, and *H. influenzae*, indicated 100% specificity of the assay. Thus, in a tertiary referral setting in southern Vietnam, the positive and negative predictive values of the test are 100%. Results of real-time PCR for specific detection of *S. pneumoniae*, *N. meningitidis*, and *H. influenzae* on samples studied retrospectively, which had been culture positive for these pathogens, showed that bacterial DNA was still detectable in 97% of samples indicating that the bacterial DNA was not affected by storage.

Prospective evaluation showed a striking additional diagnostic value of the *S. suis* serotype 2 PCR over culture, further strengthening observations that the prevalence of *S. suis* infections among Vietnamese patients with bacterial meningitis is extremely high. The diagnostic yield increased by 84% for patients with *S. suis* serotype 2 infection when using PCR. Similar differences in detection rates between culture and PCR were found for *S. pneumoniae* and *N. meningitidis*. In large part, these differences can be explained by the use of antimicrobial agents before admission and collection of specimens. In our study, overall 63.3% of patients had received antibiotics before admission, and this proportion was significantly higher in patients with culture-negative CSF samples. Furthermore, bacterial loads as assessed by Ct values in our PCR were significantly lower in pretreated patients. However, while PCR is clearly an important tool in the diagnosis of bacterial meningitis, it should not replace Gram stain and bacterial culture, given the need of a rapid presumptive diagnosis and antimicrobial susceptibility data in the treatment of this life-threatening disease. In addition, the pathogens that can be detected using PCR generally do not cover the full spectrum of potential causes of bacterial meningitis, indicating the continued need of bacterial culture.

S. suis was detected at much higher rates than *S. pneumoniae* and *N. meningitidis* in our study population. *S. suis* is increasingly recognized as an important cause of bacterial meningitis in adults, not only in Vietnam but also in China, Thailand, Singapore, and other countries in the region, while sporadic cases are reported worldwide. Risk factors for *S. suis* infection include (occupational) exposure to pigs and pig products. Consumption of undercooked pork products is increasingly being suggested as an additional risk factor for *S. suis* infection (Wertheim et al., 2009a). The mortality of *S. suis* infection varies with the clinical presentation, with the lowest mortality in patients with meningitis and the highest mortality in those presenting with a streptococcal toxic shock-like syndrome (Wertheim et al., 2009a). Resistance to penicillin in *S. suis* is extremely rare. All cultured strains in our study were sensitive to penicillin and ceftriaxone, which are the drugs of choice for treatment of *S. suis* meningitis. *S. suis* meningitis is commonly associated with neurologic sequelae, in particular hearing loss, which can be found in up to 60% of cases (Mai et al., 2008). To reliably determine the burden of disease caused by *S. suis* infection, while taking into account that over-the-counter sales of antimicrobial agents is common in regions where *S. suis* infections predominantly occur, the availability of sensitive and specific diagnostic tools to detect *S. suis* infections is extremely important. To our knowledge, this is the first prospective study on the molecular diagnosis of *S. suis* infections in humans.

In conclusion, we developed a highly sensitive and specific real-time PCR for detection of *S. suis* serotype 2 in CSF, which is now routinely used in a setting where human *S. suis* serotype 2 infection is endemic.

Acknowledgments

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References

Boom R, Sol C, Beld M, Weel J, Goudsmit J, Wertheim-van Dillen P (1999) Improved silica-guanidiniumthiocyanate DNA isolation procedure based on selective binding of bovine alpha-casein to silica particles. *J Clin Microbiol* 37:615–619.

Corless C, Guiver M, Borrow R, Edwards-Jones V, Fox A, Kaczmarski E (2001) Simultaneous detection of *Neisseria meningitidis*, *Haemophilus influenzae*, and *Streptococcus pneumoniae* in suspected cases of meningitis and septicemia using real-time PCR. *J Clin Microbiol* 39:1553–1558.

Donsakul K, Dejthevaporn C, Witoonpanich R (2003) *Streptococcus suis* infection: clinical features and diagnostic pitfalls. *Southeast Asian J Trop Med Public Health* 34:154–158.

Gottschalk M, Segura M, Xu J (2007) *Streptococcus suis* infections in humans: the Chinese experience and the situation in North America. *Anim Health Res Rev* 8:29–45.

Kerdsin A, Oishi K, Sripakdee S, Boonkerd N, Polwichai P, Nakamura S, Uchida R, Sawanpanyalert P, Dejsirilert S (2009) Clonal dissemination

of human isolates of *Streptococcus suis* serotype 14 in Thailand. *J Med Microbiol* 58:1508–1513.

Lutticken R, Temme N, Hahn G, Bartelheimer EW (1986) Meningitis caused by *Streptococcus suis*: case report and review of the literature. *Infection* 14:181–185.

Mai NT, Hoa NT, Nga TV, Linh LD, Chau TT, Sinh DX, Phu NH, Chuong LV, Diep TS, Campbell J, Nghia HD, Minh TN, Chau NV, de Jong MD, Chinh NT, Hien TT, Farrar J, Schultsz C (2008) *Streptococcus suis* meningitis in adults in Vietnam. *Clin Infect Dis* 46:659–667.

Nghia HD, Hoa NT, Linh le D, Campbell J, Diep TS, Chau NV, Mai NT, Hien TT, Spratt B, Farrar J, Schultsz C (2008) Human case of *Streptococcus suis* serotype 16 infection. *Emerg Infect Dis* 14:155–157.

Nguyen TH, Tran TH, Thwaites G, Ly VC, Dinh XS, Ho Dang TN, Dang QT, Nguyen DP, Nguyen HP, To SD, Nguyen VC, Nguyen MD, Campbell J, Schultsz C, Parry C, Torok ME, White N, Nguyen TC, Tran TH, Stepniewska K, Farrar JJ (2007) Dexamethasone in Vietnamese adolescents and adults with bacterial meningitis. *N Engl J Med* 357:2431–2440.

Smith HE, Damman M, van der Velde J, Wagenaar F, Wisselink HJ, Stockhofe-Zurwieden N, Smits MA (1999) Identification and characterization of the cps locus of *Streptococcus suis* serotype 2: the capsule protects against phagocytosis and is an important virulence factor. *Infect Immun* 67:1750–1756.

Smith HE, de Vries R, van't Slot R, Smits MA (2000) The cps locus of *Streptococcus suis* serotype 2: genetic determinant for the synthesis of sialic acid. *Microb Pathog* 29:127–134.

Tang J, Wang C, Feng Y, Yang W, Song H, Chen Z, Yu H, Pan X, Zhou X, Wang H, Wu B, Wang H, Zhao H, Lin Y, Yue J, Wu Z, He X, Gao F, Khan AH, Wang J, Zhao GP, Wang Y, Wang X, Chen Z, Gao GF (2006) Streptococcal toxic shock syndrome caused by *Streptococcus suis* serotype 2. *PLoS Med* 3:e151.

van Doornum GJ, Guldemeester J, Osterhaus AD, Niesters HG (2003) Diagnosing herpesvirus infections by real-time amplification and rapid culture. *J Clin Microbiol* 41:576–580.

Wertheim HF, Nghia HD, Taylor W, Schultsz C (2009a) *Streptococcus suis*: an emerging human pathogen. *Clin Infect Dis* 48:617–625.

Wertheim HF, Nguyen HN, Taylor W, Lien TT, Ngo HT, Nguyen TQ, Nguyen BN, Nguyen HH, Nguyen HM, Nguyen CT, Dao TT, Nguyen TV, Fox A, Farrar J, Schultsz C, Nguyen HD, Nguyen KV, Horby P (2009b) *Streptococcus suis*, an important cause of adult bacterial meningitis in northern Vietnam. *PloS One* 4:e5973.

Wisselink HJ, Joosten JJ, Smith HE (2002) Multiplex PCR assays for simultaneous detection of six major serotypes and two virulence-associated phenotypes of *Streptococcus suis* in tonsillar specimens from pigs. *J Clin Microbiol* 40:2922–2929.

Ye C, Zhu X, Jing H, Du H, Segura M, Zheng H, Kan B, Wang L, Bai X, Zhou Y, Cui Z, Zhang S, Jin D, Sun N, Luo X, Zhang J, Gong Z, Wang X, Wang L, Sun H, Li Z, Sun Q, Liu H, Dong B, Ke C, Yuan H, Wang H, Tian K, Wang Y, Gottschalk M, Xu J (2006) *Streptococcus suis* sequence type 7 outbreak, Sichuan, China. *Emerg Infect Dis* 12:1203–1208.

Streptococcus suis: An Emerging Human Pathogen

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Streptococcus suis **infection is acquired through exposure to contaminated pigs or pig meat. Over the past few years, the number of reported *S. suis* infections in humans has increased significantly, with most cases originating in Southeast Asia, where there is a high density of pigs. Increased awareness, improved diagnostics, and the occurrence of outbreaks have contributed to this increase. Meningitis and sepsis are the most common clinical manifestations of *S. suis* infection; hearing loss is a frequent complication. In this article, we provide an overview of the emergence and clinical manifestations of *S. suis* infection.**

Streptococcus suis is a pathogen in pigs that can cause severe systemic infection in humans [1]. *S. suis* was first reported by veterinarians in 1954, after outbreaks of meningitis, septicemia, and purulent arthritis occurred among piglets [2]. Fourteen years later, the first human *S. suis* cases were diagnosed in Denmark, and subsequently, other cases were reported in other northern European countries and Hong Kong [3–5].

The number of human *S. suis* cases reported in the literature has increased significantly over the past few years. In a review article published in 2007, 409 human *S. suis* cases were reported. At the time of writing of this article, this figure has increased to >700 cases, with most cases originating in Southeast Asia (figure 1) [1]. In this review, we discuss whether this increase in reported human *S. suis* cases is attributable to the emergence and spread of clones with increased capacity to infect humans in certain geographical areas and/or attributable to increased awareness and improved diagnostics of *S. suis* infection.

EPIDEMIOLOGY

S. suis infection in pigs is reported worldwide, from North America (United States and Canada) to South America (Brazil), Europe (United Kingdom, The Netherlands, France, Denmark, Norway, Spain, and Germany), Asia (China, Thailand, Vietnam, and Japan), Australia, and New Zealand [7]. In addition to pigs, *S. suis* can be isolated from other animals, such as ruminants, cats, dogs, deer, and horses, and is believed to be a commensal in the intestinal flora [7]. Healthy pigs can carry multiple serotypes of *S. suis* in their nasal cavities, tonsils, and upper respiratory, genital, and alimentary tracts [7–9]. Of the 35 known serotypes, only a limited number are responsible for infections in pigs, including serotypes 1–9 and 14 [10]. Serotype 2 is considered to be the most pathogenic for both humans and pigs [1]. *S. suis* is usually transmitted nasally or orally and colonizes the palatine tonsils of both clinically ill and healthy pigs. The infant piglets become infected after contact with colonized sows [11]. Rates of asymptomatic carriage may be as high as 80%, and the morbidity ranges from <1% to >50%, although it rarely exceeds 5% [7, 12].

Since the first human case in Denmark was reported, increasing numbers of human cases have been reported in many countries (figure 1) [1, 4, 13–16]. Recently, a large series of 151 *S. suis* meningitis cases was described in southern Vietnam over a 10-year period [16]. Although most reports concern sporadic cases of infection, an outbreak of *S. suis* infection occurred in Sichuan Province, China, during July and August 2005, that involved 215 cases and 38 deaths, emphasizing the importance of *S. suis* as an emerging zoonosis [17]. In contrast to the serotypes infecting pigs, *S. suis* serotype 2 is the most common cause of this disease in humans [1]. Serotypes 1, 4, 14, and 16 have caused severe disease in a limited number of persons [18].

Human *S. suis* infections are most often reported from coun-

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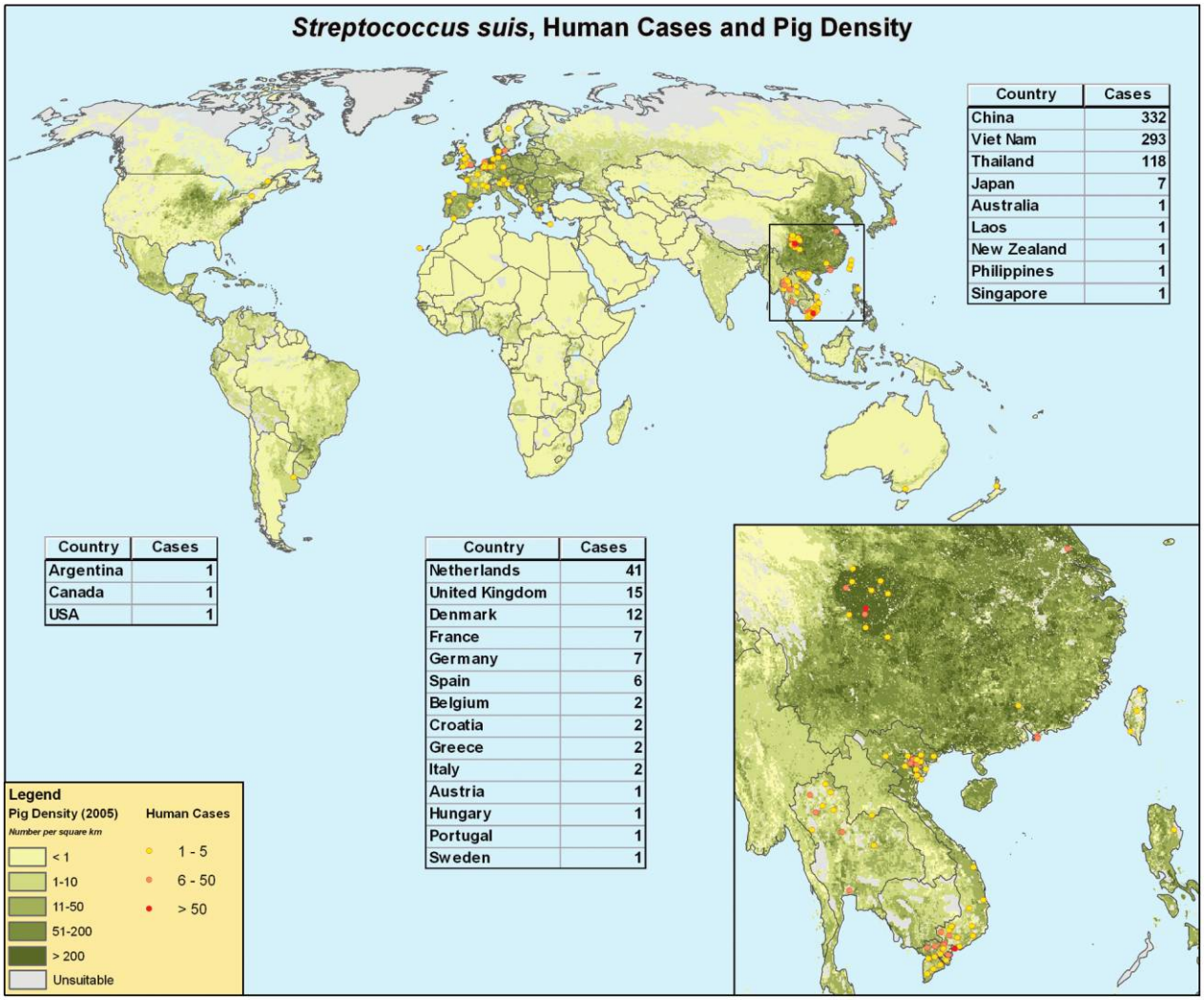

Figure 1. World map of human *Streptococcus suis* cases with background pig density data. Published with permission from the Infectious Diseases Research Foundation (World Atlas of Infectious Diseases Project) [6].

tries where pig-rearing is common (figure 1). The relative high mean patient age (47–55 years) and almost complete absence of children in case series, as well as the high male-to-female patient ratio (3.5:1.0 to 6.5:1.0) (table 1), support the notion that infection with *S. suis* is generally an occupational disease [4, 12, 13, 16, 17, 19]. The annual risk of developing *S. suis* meningitis among abattoir workers and pig breeders has been estimated to be 3.0 cases per 100,000 population; the risk is lower for butchers, at 1.2 cases per 100,000 population in developed countries [4]. Such an estimate has not been made for Southeast Asia, where the pig density is high (figure 1).

In a matched case-control study of risk factors for human *S. suis* infection in Sichuan Province, slaughtering (OR, 11.9; 95% CI, 3.4–42.8) and cutting carcasses and processing sick or dead pigs (OR, 3.0; 95% CI, 1.0–8.8) were important risk factors for human infection [20]. Farmers often share accommodations with pigs, and it is common practice for diseased animals to be slaughtered at home and consumed [11]. However, occupational or household exposure to pigs or pork is not present in all cases of *S. suis* infection. Case series from Hong Kong and Vietnam included not only a significant number of housewives, presumably infected as a result of contact with (contaminated) pork [12, 16, 21], but also other individuals who were unaware of any exposure to pork. In Vietnam, pork is the most important meat source, with >98% of households consuming pork [22]. Vietnamese consumers prefer to buy fresh pork from wet markets [22]. Because *S. suis* was isolated from 6.1% of raw pork meat from 3 of the 6 wet markets in Hong Kong, it is likely that, besides occupational exposure, processing or consuming uncooked or partially

Table 1. Features of *Streptococcus suis* infection reported from published clinical case series.

Variable	Vietnam (n = 151)	China (n = 204)	Thailand (n = 32)	The Netherlands (n = 30)
Demographic characteristic				
Male sex	117 (77.5)	171 (83.8)	23 (71.9)	26 (86.7)
Age	46.5 years (19–84 years)	54 years (NA)	49 years (1 month to 75 years)	49 years (26–76 years)
Clinical signs				
Duration of illness, days	4 (1–21)	NA	4.5 (1–14)	2 (1–5)
Fever	151 (100)	204 (100)	NA	NA
Headache	142 (94.0)	164 (80.4)	NA	NA
Vomiting	NA	117 (57.4)	NA	NA
Glasgow Coma Scale	12 (5–15)	NA	NA	NA
Stiff neck	142 (94.0)	NA	NA	NA
Skin findings	9 (6.0)	56 (27.5)	8 (25.0)	5 (16.7)
CSF findings				
Total WBC count, cells/μL	2100 (1–64,000)	NA	925 (0–21,800)	1500 (50–110,000)
Percentage of neutrophils	84 (1–99)	NA	65 (0–99)	NA
Total protein level, g/dL	2.06 (0.2–10.19)	NA	1.76 (0.75–4.56)	3 (0.8–9.8)
Glucose level, g/dL	NA	NA	5 (0–67)	27 (1.8–58.5)
Lactate level, mmol/L	11.2 (2–17)	NA	NA	NA
CSF:blood glucose level, %	13.76 (0.7–71)	NA	NA	NA
Blood findings				
Total WBC count, cells/μL	16.8 (3.8–57.0)	14.3 (9.4–31.1)	16.4 (5.6–47.3)	NA
Platelet count, cells/μL	159 (18–933)	NA	224 (22.9–515)	NA
Outcome				
Duration of hospital admission, days	14 (1–43)	15.1[a]	NA	NA
Died in hospital	4 (2.6)	38 (18.6)	2 (6.3)	2 (6.7)
Hearing loss at hospital discharge	93/140 (66.4)	NA	22/32 (68.8)	15/28 (54)

NOTE. Data are no. (%) of patients or median value (range). Data for Vietnam are from Mai et al. [16], data for China are from Tang et al. [33], data for Thailand are from Suankratay et al. [35] , and data for The Netherlands are from Arends et al. [4]. NA, not available.

[a] Estimated value.

cooked pork products is also a risk factor for infection. Local delicacies, such as undercooked pig tonsils, intestines, or uterus and fresh pig blood, may also represent important sources of infection.

The incubation period of *S. suis* infection in 1 Chinese outbreak ranged from 3 h to 14 days (median, 2.2 days) [17]. A very short incubation time is consistent with direct entry of *S. suis* into the blood through skin wounds [17]. Other researchers have reported incubation periods varying from 60 h to 1 week [4, 16]. Patients have generally been healthy prior to infection with *S. suis,* although predisposing factors, such as splenectomy, diabetes mellitus, alcoholism, malignancy, and structural heart diseases have been reported [9, 12]. Whether *S. suis* infection has a seasonal variation remains unclear. In China and Thailand, more patients were admitted during the rainy season, from June through September, than during the rest of the year [9, 12, 13]. In northern Vietnam, more cases were reported during the warmer months, from April through October, than during the rest of the year (unpublished data). In southern Vietnam, where there are fewer climatological differences throughout the year than in northern Vietnam, this distribution of cases was not as clear [16].

Although asymptomatic carriage is common in healthy pigs, it is unknown whether human carriage of *S. suis* is common. A seroprevalence study in The Netherlands revealed that 6% of the veterinarians and 1% of the pig farmers had antibody titers against *S. suis* serotype 2 antigen [23]. Nasopharyngeal carriage of *S. suis* was studied in a group at high risk of infection (butchers, abattoir workers, and meat-processing employees) in Germany. The authors reported a carriage rate of 7 (5.3%) of 132 persons, although none of the 130 control subjects, who had no contact with pigs or pork, were carriers [24]; these findings indicate that *S. suis* carriage does occur in individuals with prolonged and recurrent exposure to pigs and pork. The prevalence, duration, and importance of *S. suis* carriage in humans are unknown.

CLINICAL FEATURES

S. suis causes a systemic infection in humans that affects several organ systems; meningitis is the most common clinical manifestation [4, 13, 17]. The presenting features of *S. suis* meningitis are generally similar to those of other bacterial pyogenic meningitis and include headache, fever, vomiting, and menin-

geal signs. The duration of illness before hospital admission was 2–5 days [16, 25]. One striking feature is subjective hearing loss, which may be reported by up to one-half of patients at presentation or a few days later [9]. Six percent to 31% of patients also have skin findings, including petechiae, purpura, and ecchymoses, all of which can be extensive, and hemorrhagic bullae and skin necrosis (features of purpura fulminans) (figure 2). Gangrene of the fingers and toes may also be seen in a minority of patients at a later stage in the disease [16]. Less common manifestations of *S. suis* infection include acute and subacute endocarditis [26–28], acute pyogenic arthritis [28], endophthalmitis and uveitis [5, 9], spondylodiscitis [29], brain stem ophthalmoplegia [30], and epidural abscess [31]. Of importance, infective endocarditis was reported to be more common than meningitis in Chiang Mai, Thailand [13].

Meningitis is often accompanied by bacteremia, similar to *Streptococcus pneumoniae* and *Neisseria meningitidis* meningitis [32]. *S. suis* infection can be complicated by acute renal failure requiring renal replacement therapy, acute respiratory distress syndrome necessitating ventilation, and consumptive coagulopathy [1]. Recent outbreaks of *S. suis* infection in China have highlighted the importance and relative high frequency of a

Figure 2. Skin findings in a patient with *Streptococcus suis* meningitis and septicemia

severe sepsis syndrome—with some features suggestive of toxic shock syndrome—associated with a high mortality [17, 33]. The investigators observed erythematous blanching rash on the extremities, including blood spots and petechia, indicative of possible toxic shock syndrome [33]. However, no streptococcal superantigen was found on microbial analyses [33, 34]. Further investigations revealed that a pathogenicity island in the bacterial genome may be responsible for the more-severe *S. suis* cases that were found in China [34]. The exact role of this pathogenicity island is poorly understood, and it remains to be seen whether it has a function in *S. suis* virulence.

Hearing loss in *S. suis* meningitis is sensineural, is in the high frequency range [35], and can be profound; it was >80 dB on audiometric testing of Vietnamese adults [16]. The prognosis for hearing is guarded; some patients improve over time, and others do not. Among the Vietnamese adults, 93 (66.4%) of 140 evaluated patients had mild-to-severe hearing loss at hospital discharge, compared with 41 (47.7%) of 86 patients evaluated at 6 months after hospital discharge (table 1) [16]. Vestibular dysfunction (e.g., ataxia) has also been described in association with hearing loss [36].

MICROBIOLOGICAL DIAGNOSIS

S. suis is a gram-positive coccus that is frequently seen in pairs but can also be single or in short chains (figure 3). Determination of *S. suis* to the species level can be performed with biochemical tests, such as optochin, Voges-Proskauer, salicin, trehalose, and 6.5% sodium chloride [37]. Commercial systems (e.g., API Strep; Biomerieux) can also be used. Some commercial biochemical identification systems also report whether *S. suis* is biotype I or II. This should not be mistaken for serotype 1 or 2, as was done in some reports [38, 39]. The 35 *S. suis* serotypes can be identified by agglutination with a panel of antiserum samples. Serotyping is performed in reference laboratories.

Although *S. suis* can be cultured from CSF or blood samples with use of standard microbiological techniques, it is often misidentified, or infection goes undiagnosed [40, 41]. In our experience and others' experience, *S. suis* has been commonly misidentified and reported as *Streptococcus* species, α-hemolytic or viridans streptococci, *Enterococcus faecalis, Aerococcus viridans,* or even *S. pneumoniae* [36, 41].

Culture results can be negative as a result of, for example, antibiotic use prior to the obtainment of specimens. Molecular techniques with a *S. suis* serotype 2–specific PCR have improved the detection of *S. suis* cases in Asia, with the *cps2J* gene as a target [16, 42]. In a case series of 151 patients in Ho Chi Minh City, Vietnam, 117 were detected to have *S. suis* infection by CSF culture, and 149 had *S. suis* detected by real-time PCR. Cases in 22 patients would have remained undiagnosed if PCR was not available. After 1 week of treatment, *S. suis* DNA could still be detected by PCR in the majority of the patients' samples [16]. The implementation of molecular techniques is not always feasible in regions where *S. suis* is highly prevalent. However, great improvements can be achieved by simple microbiological capacity building. As a rule of thumb, clinicians and microbiology laboratories in regions with pig farming industries should consider *S. suis* if optochin-resistant streptococci are cultured from a CSF sample obtained from a patient with meningitis (figure 3). The identification of α-hemolytic streptococci other than *Pneumococcus* species, cultured from a CSF sample, requires further microbiological investigation, because this may be *S. suis.*

The genetic diversity of *S. suis* serotype 2 strains has been studied using various typing techniques, including random amplification of polymorphic DNA, PFGE, and ribotyping, but with the exception of the Vietnamese strain collection, the number of human strains studied to date outside outbreak situations, has been small (range, 1–27 strains), and limited epidemiological data are available [43–47]. Analysis of Vietnamese strains with use of multilocus sequence typing [48] identified 98% of the strains as sequence type 1 and belonging to clonal complex 1. The strain that caused an outbreak of infection in pigs and humans in China in 2005 was sequence type 7 and also belonged to clonal complex 1 [49]. Although strains of clonal complex 1 thus appear to be important in human infection, serotype 2 strains of clonal complex 27 were recently described to cause infections in humans in Thailand [47], which suggests that the *S. suis* population infecting humans may vary by geographical region, similar to and potentially reflecting the situation in pigs.

VIRULENCE

Little is known about how *S. suis* invades the host and how it crosses the blood brain barrier. Inflammation is likely to contribute to the manifestations of disease in pigs and probably also in humans, as has been summarized elsewhere [10]. A series of potential virulence factors of *S. suis* serotype 2 have been identified, including the capsular polysaccharide, extracellular protein factor, muramidase-released protein, suilysin, several adhesins, hyaluronate lyase, and surface antigen 1 [50–58]. With exception of the capsular polysaccharide, none of these were shown to be essential for virulence in animal models of infection, and the presence of extracellular protein factor and muramidase-released protein varied among human isolates [16, 59]. Two Chinese outbreak isolates were fully sequenced, and a proposed pathogenicity island was identified that may have been involved in the particular clinical manifestations observed during the outbreak in 2005 [34]. This pathogenicity island contains a 2-component regulatory system that may be

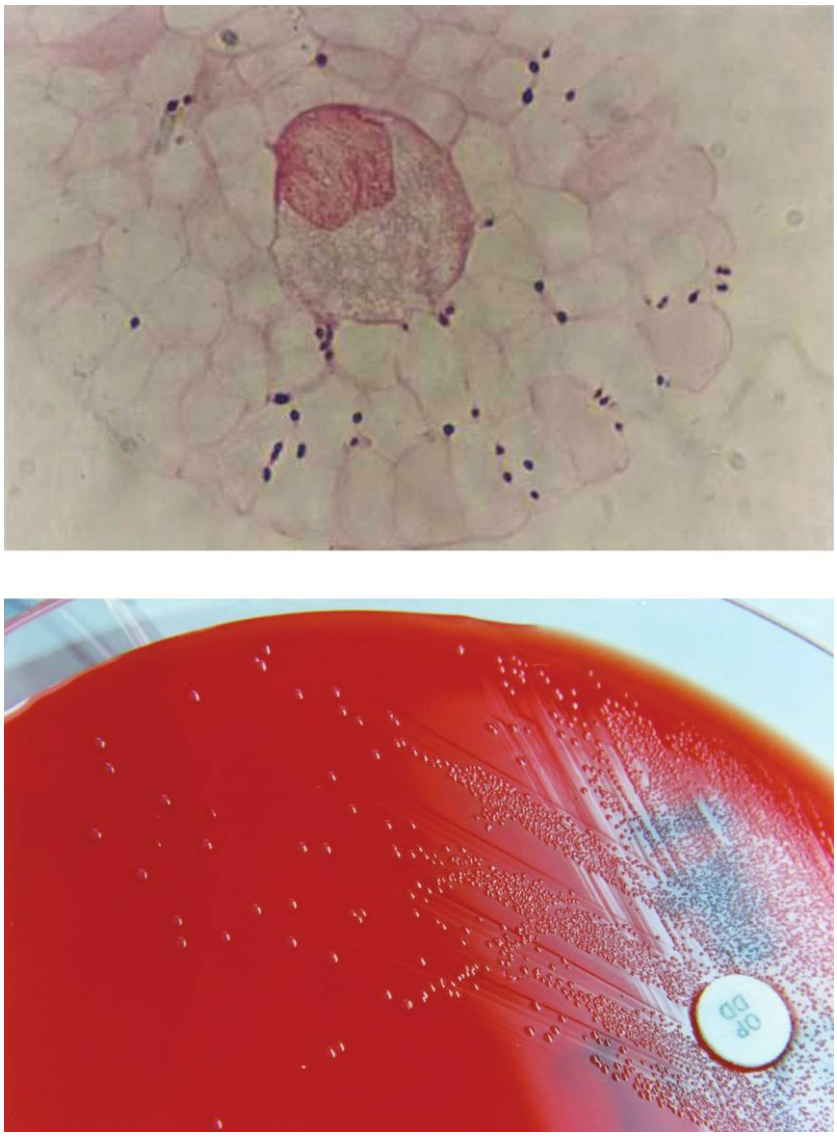

Figure 3. Gram-positive cocci *(Streptococcus suis),* single or in pairs, visible in direct Gram stain of a CSF sample (original magnification, ×100; *top).* Growth of *S. suis* colonies on blood agar with optochin disk *(bottom).*

involved in virulence [60]. More research is needed to elucidate virulence in *S. suis*.

TREATMENT AND OUTCOME

Similar to the protocol for any other patient with suspected bacterial meningitis, antibiotic treatment should be started without delay. Data from Vietnam show that *S. suis* is susceptible to penicillin, ceftriaxone, and vancomycin [16]. Resistance was seen to tetracyclin (83.2% of isolates; MIC_{50}, 16 μg/mL; MIC_{90}, 32 μg/mL), erythromycin (20%; MIC_{50}, 0.064 μg/mL; MIC_{90}, 1256 μg/mL), and cloramphenicol (3.3%) [16]. Penicillin resistance has been reported in a single human case and in some pig isolates [61, 62]. In 1 European study, the drug susceptibility of 384 *S. suis* strains from diseased pigs was assessed. The strains were susceptible to penicillin (MIC_{90}, ≤0.13 μg/mL). Low rates of resistance were observed for gentamicin (1.3% of isolates; MIC_{90}, 8 μg/mL) and trimethoprim-sulfamethoxazole (6.0%; MIC_{90}, 2 μg/mL), and a high rate of resistance was seen for tetracycline (75.1%; MIC_{90}, 64 μg/mL) [63].

The principles of treatment are the same as those for other causes of bacterial meningitis. For empirical treatment, cef-

triaxone with or without vancomycin (depending on the local epidemiology of bacterial meningitis and drug resistance) is a good choice until the diagnosis is laboratory confirmed. The same treatment dose and duration that is used for pneumococcal meningitis is also recommended for *S. suis* meningitis (i.e., ceftriaxone [2 g every 12 h for 14 days for adults]). This has achieved a high cure rate of 97% [16, 64]. Penicillin G (24 million U over 24 h for at least 10 days) has been used successfully for the treatment of *S. suis* meningitis [65]. Clinical experience with other drugs (e.g., in instances of penicillin allergy) is limited. Failure to improve during treatment or the development of a relapse should prompt a reevaluation, including a search for an intracranial abscess, a metastatic infection, a hospital-acquired infection, or the development of drug resistance. Some patients with *S. suis* meningitis have experienced relapse after 2 weeks of treatment with penicillin or ceftriaxone but responded to prolonged treatment (4–6 weeks) [10]. It must be stressed that the treatment recommendations may not be successful for all patients and may need to be tailored. For instance, in case of *S. suis* infection involving sites other than the CNS, such as endocarditis, endophthalmitis, or arthritis, the recommended guidelines should be observed in terms of duration of treatment, monitoring, and surgical intervention. There are no clinical data on the treatment of penicillin- or ceftriaxone-resistant *S. suis* infections.

The use of dexamethasone as an adjunctive treatment to reduce mortality and improve the outcome of bacterial meningitis remains controversial [66]. In a randomized, double-blind, placebo-controlled clinical trial in Vietnam, dexamethasone (0.4 mg/kg twice daily for 4 days) resulted in a significant reduction in the risk of death at 1 month (relative risk, 0.43; 95% CI, 0.20–0.94) and in the risk of death and disability at 6 months (OR, 0.56; 95% CI, 0.32–0.98) in patients with confirmed bacterial meningitis [64]. A significant proportion of these patients were infected with *S. suis* [16, 64]. Of importance, in the group of patients with *S. suis* meningitis, 20 (37.7%) of 53 patients who were given placebo were deaf in at least 1 ear, compared with 7 (12.3%) of 57 patients who were given dexamethasone ($P = .003$) [16, 64]. In a multivariate analysis, severe hearing loss was associated with older age (>50 years) and not receiving corticosteroid treatment [16]. Dexamethasone (0.4 mg/kg twice daily for 4 days) is now given to adult patients in southern Vietnam who have a short disease onset (<7 days), cloudy CSF, WBC count >1000 cells/μL (with >60% neutrophils), high CSF lactate level (>4 mmol/L), and low CSF glucose level (<50% of blood glucose) or to patients who have a positive result of at least 1 of the following tests: Gram stain, bacterial culture, bacterial antigen test, or PCR.

The reported case-fatality rates associated with *S. suis* meningitis vary and have generally been low in several meningitis series, compared with rates among patients in the same age group with meningitis due to *S. pneumoniae* and other bacterial agents (e.g., 2.6% in southern Vietnam [16] and 7% in The Netherlands [4]). An outbreak in China was associated with an overall case-fatality rate of 18%, but this reached 63% among patients with septicemia and septic shock [33].

CONCLUSIONS

The majority of the reported human *S. suis* cases originate from Southeast Asia, where the disease can be considered endemic. This finding can be explained by the high density of pigs in the region, slaughtering practices without preventive measures, and the consumption of uncooked or lightly cooked pig products. Currently, a human vaccine is not available, but simple preventive measures, such as wearing gloves during processing pig meat or slaughtering, hand washing after handling raw pork meat, and thorough cooking of pork, should prevent the majority of cases. Travelers should be aware that dietary habits in some countries may pose a risk for infectious diseases, including *S. suis* infection. A case-control study to identify risk factors for *S. suis* infection is lacking and is urgently required, because *S. suis* meningitis is one of the most common causes of adult meningitis in China, northern Thailand, and Vietnam.

Mapping of pig density and human *S. suis* cases clearly suggests where *S. suis* is likely to be present but, thus far, has not been reported. Increased awareness of both clinicians and microbiologists is needed to fully appreciate the importance of *S. suis* as a human pathogen.

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References

1. Lun ZR, Wang QP, Chen XG, Li AX, and Zhu XQ. *Streptococcus suis:* an emerging zoonotic pathogen. Lancet Infect Dis **2007**; 7:201–9.
2. Field HI, Buntain D, Done JT. Studies on pig mortality. I. Streptococcal meningitis and arthritis. Vet Rec **1954**; 66:453–5.
3. Perch B, Kristjansen P, Skadhauge K. Group R streptococci pathogenic for man: two cases of meningitis and one fatal case of sepsis. Acta Pathol Microbiol Scand **1968**; 74:69–76.
4. Arends JP, Zanen HC. Meningitis caused by *Streptococcus suis* in humans. Rev Infect Dis **1988**; 10:131–7.
5. McLendon BF, Bron AJ, Mitchell CJ. *Streptococcus suis* type II (group R) as a cause of endophthalmitis. Br J Ophthalmol **1978**; 62:729–31.
6. GeoNetwork. Food and Agricultural Organization. **2005**. Available at: http://www.fao.org/geonetwork/srv/en/metadata.show?id = 12719&currTab = distribution. Accessed 15 August 2008.

7. Staats JJ, Feder I, Okwumabua O, Chengappa MM. *Streptococcus suis:* past and present. Vet Res Commun **1997**; 21:381–407.
8. Marois C, Le Devendec L, Gottschalk M, Kobisch M. Detection and molecular typing of *Streptococcus suis* in tonsils from live pigs in France. Can J Vet Res **2007**; 71:14–22.
9. Huang YT, Teng LJ, Ho SW, Hsueh PR. *Streptococcus suis* infection. J Microbiol Immunol Infect **2005**; 38:306–13.
10. Gottschalk M, Segura M, Xu J. *Streptococcus suis* infections in humans: the Chinese experience and the situation in North America. Anim Health Res Rev **2007**; 8:29–45.
11. Sriskandan S, Slater JD. Invasive disease and toxic shock due to zoonotic *Streptococcus suis:* an emerging infection in the East? PLoS Med **2006**; 3:e187.
12. Kay R, Cheng AF, Tse CY. *Streptococcus suis* infection in Hong Kong. QJM **1995**; 88:39–47.
13. Wangkaew S, Chaiwarith R, Tharavichitkul P, Supparatpinyo K. *Streptococcus suis* infection: a series of 41 cases from Chiang Mai University Hospital. J Infect **2006**; 52:455–60.
14. Watkins EJ, Brooksby P, Schweiger MS, Enright SM. Septicaemia in a pig-farm worker. Lancet **2001**; 357:38.
15. Willenburg KS, Sentochnik DE, Zadoks RN. Human *Streptococcus suis* meningitis in the United States. N Engl J Med **2006**; 354:1325.
16. Mai NT, Hoa NT, Nga TV, et al. *Streptococcus suis* meningitis in adults in Vietnam. Clin Infect Dis **2008**; 46:659–67.
17. Yu H, Jing H, Chen Z, et al. Human *Streptococcus suis* outbreak, Sichuan, China. Emerg Infect Dis **2006**; 12:914–20.
18. Nghia HDT, Hoa NT, Linh le D, et al. Human case of *Streptococcus suis* serotype 16 infection. Emerg Infect Dis **2008**; 14:155–7.
19. Walsh B, Williams AE, Satsangi J. *Streptococcus suis* type 2: pathogenesis and clinical disease. Rev Med Microbiol **1992**; 3:65–71.
20. Yu HJ, Liu XC, Wang SW, et al. Matched case-control study for risk factors of human *Streptococcus suis* infection in Sichuan Province, China. Zhonghua Liu Xing Bing Xue Za Zhi **2005**; 26:636–9.
21. Ip M, Fung KS, Chi F, et al. *Streptococcus suis* in Hong Kong. Diagn Microbiol Infect Dis **2007**; 57:15–20.
22. Dinh XT, Nguyen TT, Tran C. Current status and prospects for the pig sector in Vietnam: a desk study. Research Report from Pro-Poor Livestock Policy Initiative. Hanoi, Vietnam: Pro-Poor Livestock Policy Initiative, **2005**. Available at: http://www.fao.org/ag/againfo/programmes/en/pplpi/docarc/rep-psd_pigmarkets_desk.pdf. Accessed 8 July 2007.
23. Elbers AR, Vecht U, Osterhaus AD, et al. Low prevalence of antibodies against the zoonotic agents *Brucella abortus, Leptospira* spp., *Streptococcus suis* serotype II, hantavirus, and lymphocytic choriomeningitis virus among veterinarians and pig farmers in the southern part of The Netherlands. Vet Q **1999**; 21:50–4.
24. Strangmann E, Froleke H, Kohse KP. Septic shock caused by *Streptococcus suis:* case report and investigation of a risk group. Int J Hyg Environ Health **2002**; 205:385–92.
25. Lun S, Willson PJ. Putative mannose-specific phosphotransferase system component IID represses expression of suilysin in serotype 2 *Streptococcus suis.* Vet Microbiol **2005**; 105:169–80.
26. Vilaichone RK, Vilaichone W, Nunthapisud P, Wilde H. *Streptococcus suis* infection in Thailand. J Med Assoc Thai **2002**; 85(Suppl 1):S109–17.
27. Voutsadakis IA. *Streptococcus suis* endocarditis and colon carcinoma: a case report. Clin Colorectal Cancer **2006**; 6:226–8.
28. Doube A, Calin A. Bacterial endocarditis presenting as acute monoarthritis. Ann Rheum Dis **1988**; 47:598–9.
29. Tsai HC, Lee SS, Wann SR, Huang TS, Chen YS, Liu YC. *Streptococcus suis* meningitis with ventriculoperitoneal shunt infection and spondylodiscitis. J Formos Med Assoc **2005**; 104:948–50.
30. Meecham JS, Worth RC. Persistent diplopia following *Streptococcus suis* type 2 meningitis. J R Soc Med **1992**; 85:579–80.
31. Ibaraki M, Fujita N, Tada M, Ohtaki O, Nagai H. A Japanese case of *Streptococcus suis* meningitis associated with lumbar epidural abscess. Rinsho Shinkeigaku **2003**; 43:176–9.
32. Dupas D, Vignon M, Geraut C. *Streptococcus suis* meningitis: a severe noncompensated occupational disease. J Occup Med **1992**; 34:1102–5.
33. Tang J, Wang C, Feng Y, et al. Streptococcal toxic shock syndrome caused by *Streptococcus suis* serotype 2. PLoS Med **2006**; 3:e151.
34. Chen C, Tang J, Dong W, et al. A glimpse of streptococcal toxic shock syndrome from comparative genomics of *S. suis* 2 Chinese isolates. PLoS ONE **2007**; 2:e315.
35. Suankratay C, Intalapaporn P, Nunthapisud P, Arunyingmongkol K, Wilde H. *Streptococcus suis* meningitis in Thailand. Southeast Asian J Trop Med Public Health **2004**; 35:868–76.
36. Donsakul K, Dejthevaporn C, Witoonpanich R. *Streptococcus suis* infection: clinical features and diagnostic pitfalls. Southeast Asian J Trop Med Public Health **2003**; 34:154–8.
37. Higgins R, Gottschalk M. An update on *Streptococcus suis* identification. J Vet Diagn Invest **1990**; 2:249–52.
38. Lee GT, Chiu CY, Haller BL, Denn PM, Hall CS, Gerberding JL. *Streptococcus suis* meningitis, United States. Emerg Infect Dis **2008**; 14:183–5.
39. Kopic J, Paradzik MT, Pandak N. *Streptococcus suis* infection as a cause of severe illness: 2 cases from Croatia. Scand J Infect Dis **2002**; 34: 683–4.
40. Gottschalk M. Porcine *Streptococcus suis* strains as potential sources of infections in humans: an underdiagnosed problem in North America? J Swine Health Production **2004**; 12:197–9.
41. Lutticken R, Temme N, Hahn G, Bartelheimer EW. Meningitis caused by *Streptococcus suis:* case report and review of the literature. Infection **1986**; 14:181–5.
42. Matsuo H, Sakamoto S. Purulent meningitis caused by *Streptococcus suis* in a pig breeder. Kansenshogaku Zasshi **2003**; 77:340–2.
43. Berthelot-Herault F, Marois C, Gottschalk M, Kobisch M. Genetic diversity of *Streptococcus suis* strains isolated from pigs and humans as revealed by pulsed-field gel electrophoresis. J Clin Microbiol **2002**; 40: 615–9.
44. Chatellier S, Gottschalk M, Higgins R, Brousseau R, Harel J. Relatedness of *Streptococcus suis* serotype 2 isolates from different geographic origins as evaluated by molecular fingerprinting and phenotyping. J Clin Microbiol **1999**; 37:362–6.
45. Hampson DJ, Trott DJ, Clarke IL, Mwaniki CG, Robertson ID. Population structure of Australian isolates of *Streptococcus suis.* J Clin Microbiol **1993**; 31:2895–900.
46. Luey CK, Chu YW, Cheung TK, et al. Rapid pulsed-field gel electrophoresis protocol for subtyping of *Streptococcus suis* serotype 2. J Microbiol Methods **2007**; 68:648–50.
47. Takamatsu D, Wongsawan K, Osaki M, et al. *Streptococcus suis* in humans, Thailand. Emerg Infect Dis **2008**; 14:181–3.
48. King SJ, Leigh JA, Heath PJ, et al. Development of a multilocus sequence typing scheme for the pig pathogen *Streptococcus suis:* identification of virulent clones and potential capsular serotype exchange. J Clin Microbiol **2002**; 40:3671–80.
49. Ye C, Zhu X, Jing H, et al. *Streptococcus suis* sequence type 7 outbreak, Sichuan, China. Emerg Infect Dis **2006**; 12:1203–8.
50. Brassard J, Gottschalk M, Quessy S. Cloning and purification of the *Streptococcus suis* serotype 2 glyceraldehyde-3-phosphate dehydrogenase and its involvement as an adhesin. Vet Microbiol **2004**; 102:87–94.
51. de Greeff A, Buys H, Verhaar R, Dijkstra J, van Alphen L, Smith HE. Contribution of fibronectin-binding protein to pathogenesis of *Streptococcus suis* serotype 2. Infect Immun **2002**; 70:1319–25.
52. Gottschalk M, Segura M. The pathogenesis of the meningitis caused by *Streptococcus suis:* the unresolved questions. Vet Microbiol **2000**; 76:259–72.
53. King SJ, Allen AG, Maskell DJ, Dowson CG, Whatmore AM. Distribution, genetic diversity, and variable expression of the gene encoding hyaluronate lyase within the *Streptococcus suis* population. J Bacteriol **2004**; 186:4740–7.
54. King SJ, Heath PJ, Luque I, Tarradas C, Dowson CG, Whatmore AM. Distribution and genetic diversity of suilysin in *Streptococcus suis* isolated from different diseases of pigs and characterization of the genetic basis of suilysin absence. Infect Immun **2001**; 69:7572–82.

55. Li Y, Martinez G, Gottschalk M, et al. Identification of a surface protein of *Streptococcus suis* and evaluation of its immunogenic and protective capacity in pigs. Infect Immun **2006**; 74:305–12.
56. Smith HE, Wisselink HJ, Stockhofe-Zurwieden N, Vecht U, Smits MM. Virulence markers of *Streptococcus suis* type 1 and 2. Adv Exp Med Biol **1997**; 418:651–5.
57. Tikkanen K, Haataja S, Finne J. The galactosyl-(alpha 1–4)-galactose-binding adhesin of *Streptococcus suis:* occurrence in strains of different hemagglutination activities and induction of opsonic antibodies. Infect Immun **1996**; 64:3659–65.
58. Winterhoff N, Goethe R, Gruening P, et al. Identification and characterization of two temperature-induced surface-associated proteins of *Streptococcus suis* with high homologies to members of the Arginine Deiminase system of *Streptococcus pyogenes.* J Bacteriol **2002**; 184: 6768–76.
59. Takamatsu D, Osaki M, Tharavichitkul P, Takai S, Sekizaki T. Allelic variation and prevalence of serum opacity factor among the *Streptococcus suis* population. J Med Microbiol **2008**; 57:488–94.
60. Li M, Wang C, Feng Y, et al. SalK/SalR, a two-component signal transduction system, is essential for full virulence of highly invasive *Streptococcus suis* serotype 2. PLoS ONE **2008**; 3:e2080.
61. Shneerson JM, Chattopadhyay B, Murphy MFG, Fawcett IW. Permanent perceptive deafness due to *Streptococcus suis* type-II infection. J Laryngol Otol **1980**; 94:425–7.
62. Marie J, Morvan H, Berthelot-Herault F, et al. Antimicrobial susceptibility of *Streptococcus suis* isolated from swine in France and from humans in different countries between 1996 and 2000. J Antimicrob Chemother **2002**; 50:201–9.
63. Wisselink HJ, Veldman KT, Van den Eede C, Salmon SA, Mevius DJ. Quantitative susceptibility of *Streptococcus suis* strains isolated from diseased pigs in seven European countries to antimicrobial agents licensed in veterinary medicine. Vet Microbiol **2006**; 113:73–82.
64. Nguyen TH, Tran TH, Thwaites G, et al. Dexamethasone in Vietnamese adolescents and adults with bacterial meningitis. N Engl J Med **2007**; 357:2431–40.
65. Halaby T, Hoitsma E, Hupperts R, Spanjaard L, Luirink M, Jacobs J. *Streptococcus suis* meningitis, a poacher's risk. Eur J Clin Microbiol Infect Dis **2000**; 19:943–5.
66. Greenwood BM. Corticosteroids for acute bacterial meningitis. N Engl J Med **2007**; 357:2507–9.